ON ANAPHYLATOXINS AND ENDOTOXINS OF THE TYPHOID BACILLUS.*

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The endotoxin theory of Pfeiffer, although failing to explain the systemic symptoms of intoxication accompanying diseases like those caused by anthrax bacilli, streptococci, staphylococci, and some other bacteria from the bodies of which no poisonous substances could be obtained, nevertheless furnished an explanation, satisfactory until recently, for the general toxemia accompanying typhoid fever, cholera, and a number of other infections.

In the latter conditions the fact that the injection of dead bacteria or of bacterial extracts, obtained in various ways, had strong toxic action, seemed to indicate the existence of preformed poisonous ingredients in the cell bodies of the bacteria.

The recent work of Friedemann (1), and especially that of Friedberger (2) and his pupils, on the production of non-specific, acutely toxic substances (anaphylatoxins) from cells and bacteria of various kinds, by treatment with specific antibody and complement, has given ample ground for questioning whether the assumption of specific endotoxins, in any of the bacteria, is at all necessary for the comprehension of the bacterial toxemias.

In the light of this work, we may justly assume that the toxic substances may appear only after proteid cleavage of the bacterial bodies has been initiated by the action upon them of the serum components, and that the apparent specificity of the poisons, or differences between the toxemic manifestations of various diseases, may depend not on differences in the pharmacological actions of these poisons, but rather upon variations in the invasive properties

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of the bacteria, both as concerns their quantitative distribution and their accumulation and localization in the infected body.

If we leave out of consideration bacteria which, like the diphtheria bacillus, produce true secretory poisons, it would be the ability to gain a foothold in the body, the degree of invasive power, the predilection in the choice of a path of entrance, and the specific local accumulation, upon which the speed and quantity of toxin production and absorption would depend, and which consequently would give character to variations in the clinical pictures of different diseases. Besides simplifying considerably our comprehension of bacterial toxemia, the point of view suggested by this work again brings out the great importance of the work of Vaughan, and Vaughan and Wheeler (3), on the non-specific poisonous fraction obtained by hydrolysis of bacterial and other proteids, and makes it desirable that the particular conditions of anaphylatoxin and endotoxin production in the case of individual pathogenic bacteria should be carefully studied.

For, even though the existence of anaphylatoxins seems to render the assumption of endotoxins unnecessary, it is still possible that such bodies may exist in addition to the anaphylatoxins, adding a further specific element to the clinical characteristics of each disease.

Though the toxic action of bacteria freshly extracted by such rapid methods as that of freezing and grinding, as practiced by MacFadyen and others, would seem actually to prove the existence of endotoxins, even this method may be interpreted merely as another way of furnishing antigen, or matrix, for anaphylatoxin, since Neufeld and Dold (4) have produced such poisons by the action of fresh serum components on prodigiosus and typhoid extracts.

The purpose of the experiments recorded in the present paper was to study particularly the conditions of anaphylatoxin production from the typhoid bacillus. They are published because, in a number of points, they show results which differ in principle from those obtained for anaphylatoxins in general by Friedberger, Neufeld and Dold, and others, and which, in the writer's opinion, rather strengthen the conception of the manifestations of typhoid fever as being largely dependent upon anaphylatoxin poisoning.

I.

The first series of experiments was carried out according to the earlier technique of Friedberger, in that the bacteria were first sensitized in the cold (3° to 5° C.) for twenty-four hours, then washed and exposed to complement at the same temperature for the same length of time. Injections were then made into the jugular veins of guinea pigs.

The particular point in this first series of experiments was to study the relationship between the degree of anaphylatoxin production and the bactericidal titre (sensitizer content) of the immune sera.

All the experiments were carried out with the same strain of typhoid bacilli (*Bacillus typhosus* 65) and the bactericidal titrations were done by a slightly modified Stern-Korte technique, with fresh rabbit serum as complement, and the usual controls.

These experiments in the main tend merely to confirm the work of Friedberger in showing that when small amounts of bacteria are employed an excess of sensitizer tends to reduce the yield of anaphylatoxin. An example of a successful experiment is given in the following protocol.

TITRATION EXPERIMENT WITH TYPHOID IMMUNE SERUM.

Rabbit 79.

Dilution of serum.	Agglutination.	Bactericidal titre with modified Stern-Korte method.
1 : 100	+++	480 colonies.
1 : 200	+++	556 colonies.
1 : 500	+++	750 colonies.
1 : 1,000	++	Over 10,000 colonies.
1 : 2,000	±	+++++
1 : 5,000	−	+++++
1 : 10,000	−	+++++

0.2 c.c. of this serum added to 1 c.c. of typhoid filtrate gave a very slight clouding in about 15 minutes.

ANAPHYLATOXIN EXPERIMENTS.

No. in series.	Typhoid bacilli.	Amount of inactive serum.	Amount of complement.	Weight of animal.	Result.
1	⅓ slant	5.0 c.c.	4 c.c.	215 gm.	Very sick, recovers.
2	⅓ slant	3.5 c.c.	4 c.c.	200 gm.	Typical death in 2 min.
3	⅓ slant	3.0 c.c.	4 c.c.	198 gm.	Typical death in 2 min.
4	⅓ slant	2.0 c.c.	4 c.c.	225 gm.	Typical death in 2 min.
5	⅓ slant	1.0 c.c.	4 c.c.	200 gm.	Sick, recovers.

The above tabulation may be taken as summarizing a considerable number of similar experiments. Throughout our work with low temperature exposures, however, fatal anaphylatoxins were obtained in a few cases only. This, taken together with the work described below, seems to indicate that, in the case of the typhoid bacillus, a more vigorous action of serum upon the bacteria than is obtained at 0° to 5° C. is necessary for the formation of these poisons.

Moreover with the small amounts of bacteria (1/5 to 1/3 agar slants) and the low temperature exposures, we succeeded in no case in producing anaphylatoxin by the action of complement alone.

Because of the weak nature of the poisons produced in most cases, it was now considered advisable to change the technique to one used by Friedberger in his later work, in which the sensitized bacteria were exposed to the action of the complement at 37.5° C. It seemed to the writer that possibly the strong inhibition of complement action by the low temperature might account for the poor yield of poisons, but the higher temperature had been avoided because of the fear that a too vigorous lytic action might prevent the production of anaphylatoxins, as indicated in the experiments of Neufeld and Dold. A chance experiment carried out at this time furnished an indication for the proper direction in which to continue the work.

In the course of experiments planned to remove sensitizer from an inactivated immune serum, the total bacteria of ten agar slants had been left in contact with two cubic centimeters of serum for one hour at 37.5° C. The mixture was then centrifugalized, the serum used for another purpose, and to the bacteria, washed once, were added four cubic centimeters of fresh guinea pig serum. These mixtures were left in the incubator over night, then centrifugalized, and the supernatant fluid was injected into guinea pigs. The experiment was done in duplicate. There were no small pigs in the laboratory at this time and animals weighing 300 and 350 grams had to be used. The results were as follows:

No. in series.	Amount of bacteria.	Immune serum 1 hr. at 37.5° C.	Complement 12 hrs. at 37.5° C.	Weight of guinea pig.	Result.
1	10 slants	2 c.c.	4 c.c.	300 gm.	Typical death in 2 min.
2	10 slants	2 c.c.	4 c.c.	350 gm.	Typical death in 4 min.

This experiment indicated definitely that, in the case of typhoid bacilli, at least, comparatively large quantities of bacteria could be used without inhibiting the production of anaphylatoxin, a result in contrast with some of Friedberger's experiments, and that exposure to conditions, under which continued action of the complement could take place for prolonged periods of time (twelve hours), did not lead to negative results.

Since Neufeld and Dold found that no anaphylatoxin was obtained when 2 c.c. of complement were allowed to act upon three loops of sensitized cholera spirilla for but one and a half hours at 37.5° C., and concluded from this that in the course of actual lysis the anaphylatoxins were either hindered in appearance or destroyed, our apparently contradictory result called for further investigation.

The fact that powerful anaphylatoxin could be produced from sensitized typhoid bacilli, even when large amounts of bacteria were used and the exposure to complement was continued for as long as twelve hours at 37.5° C., seemed to indicate that in the case of this microörganism there was either a slower, cumulative formation of the poison or that, once formed, it was more stable and not as easily further disintegrated ("abgebaut") into non-toxic substances. This would, in our opinion, tend to throw more weight upon the assumption that anaphylatoxins were responsible to a large extent for the toxemic manifestations of typhoid fever, where, as we know, considerable quantities of bacteria are exposed to the prolonged action of high concentrations of the specific antibodies at temperatures of from 37.5° to 40° C.

In order to study further these relations the following experiments were carried out. In both experiments the bacteria were grown for twenty-four hours on agar slants, taken up in salt solution, and when sensitized, washed once before the addition of complement.

Comparing the results of the experiments summarized in these two protocols with those reported in the first part of this paper we see that the exposure of bacilli to the action of serum constituents at a temperature of 37.5° C. is, at least in the case of *Bacillus typhosus,* a much more efficient and regular method of obtaining the

anaphylatoxin than is the original method of allowing this interaction to take place at low temperatures.

EXPERIMENT I.

The materials used were *Bacillus typhosus* 65, typhoid immune rabbit serum (from rabbit A)[1] inactivated at 56° C., and fresh guinea pig serum as complement. The injected guinea pigs weighed from 150 to 225 grams.

No. in series.	Amount of bacteria.	Sensitized with inactive serum 1 hr. at 37.5° C.	Time of exposure to complement at 37.5° C.	Amount injected.	Result.
1	½ slant	1 c.c.	1 hr.	4 c.c.	Very slightly sick, recovers.
2	1 slant	1 c.c.	1 hr.	4 c.c.	Distinct symptoms, recovers.
3	1 slant	Not sensitized	1 hr.	4 c.c.	Distinct symptoms, recovers.
4	1½ slants	1 c.c.	1 hr.	4 c.c.	Typical death in 2 min.
5	2 slants	1 c.c.	1 hr.	4 c.c.	Typical death in 4 min.
6	9 slants	1 c.c.	1 hr.	4 c.c.	Typical death in 5 min.
7	9 slants	Not sensitized	1 hr.	4 c.c.	Typical death in 5½ min.
8	12 slants	1 c.c.	1 hr.	4 c.c.	Very sick, recovers.
9	12 slants	Not sensitized	1 hr.	4 c.c.	Very slightly sick, recovers.

EXPERIMENT II.

The materials used and the technique of the experiment were the same as those in experiment I.

No. in series.	Amount of bacteria.	Sensitized with inactive serum 1 hr. at 37.5° C.	Time of exposure to complement at 37.5° C.	Amount injected.	Result.
1	½ slant	1 c.c.	15 hrs.	4 c.c.	Very sick, recovers.
2	1 slant	1 c.c.	15 hrs.	4 c.c.	Typical death in 8½ min.
3	2 slants	1 c.c.	15 hrs.	4 c.c.	Typical death in 5 min.
4	2 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 5½ min.
5	3 slants	1 c.c.	15 hrs.	2.5 c.c.	Typical death in 4 min.
6	3 slants	4 c.c.	15 hrs.	4 c.c.	Typical death in 4 min.
7	3 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 2 min.
8	8 slants	1 c.c.	15 hrs.	4 c.c.	Typical death in 5 min.
9	8 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 4 min.
10	12 slants	1 c.c.	15 hrs.	4 c.c.	Very sick, lives.
11	12 slants	Not sensitized	15 hrs.	4 c.c.	Slightly sick.

The most important and interesting point brought out in these two protocols, however, it seems to us, is the fact that, with approximately equal quantities of bacteria, exposure to the serum for as long as fifteen hours at 37.5° C. not only does not destroy the

[1] This serum had an agglutinating titre of 1:8,000 for *Bacillus typhosus* 65.

anaphylatoxins once formed, but gives more powerful results than in the shorter periods of exposure. Thus guinea pigs 2 and 3 of experiment I recovered, whereas guinea pig 2 of experiment II died with typical symptoms in eight and a half minutes. In this, as in subsequent work, we have found that, with typhoid bacilli, the method of prolonged exposure gives powerful poisons with unfailing regularity. In the case of *Bacillus typhosus,* therefore, the anaphylatoxins are not as unstable and amenable to destruction by serum proteolysis as in the case of some other bacteria investigated by Neufeld and Dold.

When quantities of bacteria as large as twelve slants (or more in a few untabulated experiments) were used without sensitization or with moderate sensitization, no poisons were formed. This is in accord with the experiments of Friedberger and others with shorter exposures at 37.5° C., and with the low temperature method.

Although we cannot fully explain this point, it would seem that possibly this could be taken to support the view expressed by Bordet (5) that the distribution of serum substances upon an antigen is such that the entire amount of antibody is distributed equally among the antigenic elements, and, in the case of an excess of bacteria, as in our experiments, the quantity falling to each unit is insufficient, at least in the time of exposure here practiced, to accomplish the cleavage necessary for poison production.

It would appear from these experiments, moreover, that within a range of from no sensitization to that of sensitization of three slants with four cubic centimeters of antiserum, there is no difference in anaphylatoxin production, either in the case of exposures to 37.5° C. for one hour, or in the more prolonged experiments.

This would be, if true, a contradiction of the results of Friedberger and Szymanowski's experiments with *Vibrio metchnikovi,* which showed that, while anaphylatoxins could be produced by the action of complement upon both the sensitized bacteria and the unsensitized, the process was considerably slower in the latter case. Since the experiments of these observers were carried out with quantities of four loopfuls of the vibrios, it seemed desirable that experiments should be carried out by us with typhoid bacilli in the larger quantities used in our experiments, in order that we might

determine especially whether or not the sensitizer took any part in the production of anaphylatoxin in the method pursued by us.

Experiments were done with this point in view and it was found that, when three slants of typhoid bacilli were used, fatal anaphylatoxins could be produced from sensitized bacilli in forty minutes at 37.5° C., while it required an hour or longer to procure similar results from the unsensitized bacteria. Since this merely confirms the results of Friedberger and Szymanowski the protocols of our experiments are omitted.

There is not a fundamental difference between the action of complement on a bacterial antigen with and without the coöperation of a specific antibody, if we consider that complement in small amounts may be fixed by bacterial emulsions of various kinds. Whether this is accomplished by the aid of small amounts of specific sensitizer normally present in the guinea pig serum, or by reason of peculiarities of the bacterial proteid which, differing from some other cellular antigens, is capable of adsorbing complement independently of the sensitizer, the fact remains that we have here a capacity of bacteria for entering into relationship with complement, and the addition of specific immune bodies merely increases this capacity and hastens its action. And this conception does not appear to us affected by the doubt recently thrown upon the identification of complement fixation with complement action by the work of Liefmann and Cohn (6); for while the binding of complement is there conceived not as preceding hemolysis (and by analogy bacteriolysis), but as resulting from the liberation during this process of complement-fixing products, even in such a case the actual fixation would be an indication of a reaction having taken place in which complement has actively functionated.[2]

II.

It was a part of the plan of this work to compare the poisonous properties of the typhoid anaphylatoxins prepared as described above with the toxic action of the substances obtained from typhoid bacilli by methods hitherto spoken of as endotoxin extraction. It

[2] In connection with the work reported in this paper we have attempted to repeat the experiments of Keysser and Wassermann (7) who obtained anaphyla-

seemed also important to carry out, in such a comparative study, experiments parallel to those described in the recent work of Rosenow (8), who succeeded in obtaining substances similar to anaphylatoxins in their action upon guinea pigs, from pneumococci, typhoid bacilli, and other bacteria by a process probably of autolysis.

For these reasons the following experiments were carried out in the course of our work on typhoid anaphylatoxins.

INJECTION INTO GUINEA PIGS OF WHOLE TYPHOID BACILLI KILLED AT 60° C.

The organisms, *B. typhosus* 65, were grown on agar slants. They were then taken up in salt solution, heated to 60° C. for one hour in a water bath and immediately injected intravenously. The following table is typical of the results obtained.

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	1 slant	210 gm.	No immediate symptoms; dead in 2 dys.
2	1 slant	225 gm.	No immediate symptoms; lives.
3	2 slants	195 gm.	No immediate symptoms; dead in 26 hrs.

EXPERIMENTS WITH CULTURE FILTRATES.

1. *B. typhosus* 65 was cultivated in 0.3 per cent. alkaline broth for ten days and then filtered. 5 c.c. of this filtrate injected into a rabbit of 1,350 gm. produced diarrhea (feces streaked with blood), weakness, paralysis of hind legs, and death during the night, after about 16 to 24 hours.

Experiment with Guinea Pigs.

No. in series.	Intravenous injection.	Weight of guinea pig.	Result.
1	4 c.c.	225 gm.	No symptoms after injection. Diarrhea. Dead in 48 hrs.
2	5 c.c.	250 gm.	Dead in 72 hrs.
3	5 c.c.	265 gm.	Very sick after 2 dys; recovers.

2. *B. typhosus* 65 was cultivated in alkaline broth for two months and then filtered. 5 c.c. of this filtrate produced diarrhea and slight illness in a rabbit of 1,500 gm., but did not kill it.

toxins by allowing complement to act upon kaolin and barium sulphate previously sensitized by exposure to inactivated normal and immune sera. We have so far been unable to confirm their results, being forced to attribute the moderate symptoms resulting in some of our experiments to the similar action of slight amounts of kaolin injected with the serum. Comparative titration, moreover, did not indicate that, as claimed by the above writers, kaolin emulsions may absorb sensitizer or amboceptor out of concentrated inactivated serum.

Experiment with Guinea Pigs.

No. in series.	Intravenous injection.	Weight of guinea pig.	Result.
1	5 c.c.	240 gm.	Slight immediate illness; recovery.
2	4 c.c.	180 gm.	Slight immediate illness; recovery.

EXTRACTION OF TYPHOID BACILLI BY MECHANICAL METHODS.

1. The bacilli from twelve agar slants were emulsified in 25 c.c. of salt solution, heated to 60° C. for one hour, then placed in a shaking machine in a bottle with sterile glass beads and shaken for forty-eight hours at room temperature. The emulsion was then centrifugalized until most of the bacterial bodies had been thrown down, and the supernatant fluids were injected intravenously. The results were as follows:

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	4 c.c.	200 gm.	No immediate symptoms; survives.
2	4 c.c.	200+ gm.	No immediate symptoms; survives.

2. Bacilli from twenty agar slants were taken up in 10 c.c. of salt solution and centrifugalized until almost all had been thrown down. The bulk of the sediment, having a volume of 1.5 c.c., was freed from all supernatant fluid with pieces of blotting paper, and to it was added 0.45 gm. of sodium chloride in the centrifuge tube. This formed a paste which became very thick and gritty when placed in a beaker containing a mixture of brine and ice, in which state it was ground with a glass rod for one hour. Sterile distilled water was then added to give a total volume of 50 c.c., which gave a concentration of 0.9 per cent. of salt. This was heated to 65° C. for one hour and then injected. The opalescent liquid resulting from this treatment contained many well preserved bacilli but, with them, a large number of swollen and granular, poorly staining shadow forms, indicating that many of the bacteria had become disintegrated.

The results of injection were as follows:

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	3 c.c.	200 gm.	Very sick after injection but symptoms not like those of anaphylatoxin. Cowers quietly, fur ruffled, rapid breathing. Dead in 8 hrs.
2	2 c.c.	110 gm.	Same as above. Dead in 6 hrs.

EXPERIMENTS WITH TYPHOID BACILLI SUSPENDED FOR SHORT PERIODS IN NEUTRAL OR INACTIVE LIQUIDS (AUTOLYSATES (?)).

During the course of the immunization of rabbits with typhoid bacilli, an accident occurred which, in conjunction with the work

of Neufeld and Dold, and the similar studies of Rosenow, encouraged us to investigate the toxic properties of typhoid bacillus autolysates. It had been our habit to begin the immunization of rabbits with injections of one half an agar slant of bacilli heated to 60° C. for one hour. The material for such an injection had been prepared and, because of interruption, was allowed to stand at room temperature over night, instead of being injected at once, as was usually done. On the following morning, two unusually large Belgian hares, weighing 3,000 grams, were injected intravenously and became severely sick, dying within twenty-four hours. Since much smaller rabbits had repeatedly withstood, without symptoms, these and larger doses of dead typhoid bacilli of strain 65, we were at first at a loss to explain the death of these animals, until, upon reading the experiments of the workers quoted above, the possibility of autolytically produced toxins suggested itself.

In consequence it seemed advisable, in connection with our work on anaphylatoxins, to study upon guinea pigs the poisonous properties of substances formed in salt solution emulsions of typhoid bacilli. The following protocols illustrate these experiments.

EXPERIMENT I.

B. typhosus 65, grown for twenty-four hours on agar slants, was emulsified in salt solution in proportions of one slant to 4 c.c. of salt solution. The emulsions were kept in the incubator in centrifuge tubes, and, at varying times as indicated, one of them was taken out, most of the bacilli were thrown down, and the supernatant liquid was injected intravenously into guinea pigs with the following results.

No. in series.	Length of time at 37.5° C.	Amount injected.	Weight of guinea pig.	Result.[3]
1	1 hr.	4 c.c.	200 gm.	No immediate symptoms.
2	2 hrs.	4 c.c.	195 gm.	Slight respiratory distress.
3	6 hrs.	4 c.c.	215 gm.	No symptoms.
4	8 hrs.	4 c.c.	225 gm.	Convulsive twitching, great respiratory distress, falls to side; recovers.
5	8 hrs.	4 c.c.	210 gm.	Slightly less than preceding.
6	24 hrs.	4 c.c.	250 gm.	Very slightly sick.

Experiments I and II were not carried out with the purpose of making an extensive study of the so called typhoid endotoxins.

[3] A number of these guinea pigs died later owing to injection of living bacteria incompletely thrown down in centrifugation.

EXPERIMENT II.

Four agar slants of *B. typhosus 65* were emulsified in 12 c.c. of salt solution. They were then divided into 3 parts of 4 c.c. each and each part was shaken up with 1 c.c. of ether, and placed at 37.5° C. for varying times, as indicated.

No. in series.	Length of time at 37.5° C.	Amount injected.	Weight of guinea pig.	Result.
1	2 hrs.	4 c.c.	240 gm.	Very slightly sick.
2	3 hrs.	4 c.c.	225 gm.	Very slightly sick.
3	4½ hrs.	4 c.c.	210 gm.	Very sick, convulsive movements, weak, falls on side, difficult respiration; recovers.

but simply in order to furnish a basis for comparison between the action of these bodies and the anaphylatoxic substances. In none of the animals in which death resulted from injections of the typhoid extracts were either the symptoms or the necropsies in any way comparable to those occurring in the cases of anaphylatoxin poisoning. None of the former showed the characteristic inflation of the lungs invariably found in the latter. Moreover, in animals dying of the extracts, hemorrhages into the serous surfaces, chiefly the peritoneum, and into the wall of the large intestine, were not infrequent, and these animals often had diarrhea before death. This was especially the case in animals dying of injections of the more powerful poisons produced by the modification of Besredka's method in which the bacilli had been ground with salt before extraction. Even in these cases, however, the clinical picture was very different and more protracted than in the cases of fatal anaphylatoxin poisoning.

Now it by no means follows that this is evidence of a fundamental difference between the two modes of poisoning, since the injection of any or all of the typhoid extracts might be rationally interpreted merely as the furnishing of a matrix, or antigen, for the production of anaphylatoxin by the blood constituents of the animal after their introduction.

The slower death from these extracts might then be looked upon as a gradual anaphylatoxin poisoning, progressive and cumulative, which would be likely to differ, both in symptomatology and pathology, from that of the more sudden and acutely fatal anaphylatoxin poisoning. To determine this point it would be desirable to experi-

ment upon the results of the frequent administration of small quantities of anaphylatoxin over a prolonged period. On this point we have, at present, no completed observations.

SUMMARY AND CONCLUSIONS.

The experiments recorded in this paper confirm the observations of Friedberger that acutely toxic bodies can be produced from typhoid bacilli by the action of sensitizer and complement and that, when small quantities of bacteria are used, an excess of sensitization either interferes with the formation of the poisons or leads to a cleavage of the bacterial proteid beyond the poisonous intermediate products spoken of as anaphylatoxins. Unlike the experience of other workers with poisons of this nature, however, our experiments have shown that the action of complement upon typhoid bacilli strongly sensitized or not at all sensitized may be carried on, at body temperature, for considerably longer than twelve hours without leading to a destruction of the poisons, and that this is true when the quantities of the bacteria used vary within the wide range of from one to twelve agar slants. It has been found, in fact, that in the case of this microörganism prolonged exposure at the higher temperature of considerable quantities of bacteria constitutes an unfailing method of regularly obtaining powerful poisons. The results obtained by the use of smaller quantities and the less vigorous complement action at low temperatures are far less regular or satisfactory.

It would appear from this that complement action of considerable vigor is required to obtain from this bacillus any appreciable yield of anaphylatoxin, and that the poison, once formed, is not as unstable as that found in other microörganisms by Neufeld and Dold and others. In fact, although we have never observed complete lysis *in vitro* of the typhoid bacilli treated with antibody and complement, the sensitized bacteria exposed to the action of complement for as long as fifteen hours at 37.5° C. showed, in our experiments, much disintegration, and yet powerful poisons were present.

Were the influence of lysis or of the too vigorous action of the serum bodies as rapidly poison-destroying in the case of this

bacillus as it has been shown to be in the case of some other bacteria, it would be hard to understand how anaphylatoxins could play any part in the toxemia of typhoid fever. This phase of our experiments, however, seems to indicate that the conditions prevailing in the infected body at the height of this disease would furnish ideal criteria for anaphylatoxin production, since, in such cases, vigorously sensitized bacilli, in large numbers, are under the prolonged influence of considerable quantities of complement, conditions exactly comparable to those prevailing in our experiments.

Granted that this state of affairs is actually the case, then the subsidence of the disease might depend merely upon limitation of the supply of antigen, as the increasing bactericidal action of the blood constituents come into play, and upon the consequent diminution of the anaphylatoxin. For as the bacteria diminish and the sensitizer increases, a changed proportion between them is established which, finally, as experiment has shown, results in a failure of anaphylatoxin production. For although our experiments have shown that, within a wide latitude of relative proportions of bacteria and antibody, anaphylatoxin can be formed, beyond this range an excess of one or the other element eventually will prevent their formation. It is not, however, the purpose of this paper to discuss the mechanism of the subsidence of the disease since this phase of the work will necessitate further experimental study.

In regard to the experiments with kaolin, we were unable to confirm the contention of Keysser and Wassermann, though it is more than likely that toxic bodies could be formed by the action of complement upon any foreign proteid rendered amenable to its action. We are not inclined to attribute too much importance to these negative results, recording them merely as they occurred. However, should it be found subsequently that anaphylatoxins can be formed in this way, it seems unlikely that they are formed from the sensitizer or amboceptor as matrix, since this was not specifically adsorbed out of concentrated serum by the kaolin in our experiments.

On the basis of experiments with so called endotoxins, we feel that the existence of such preformed intracellular poisons as an element in typhoid toxemia has not been proved, and is not abso-

lutely necessary for the explanation of the phenomena occurring in this disease. However, the diarrhea, the hemorrhagic lesions, and the protracted symptoms following the injection of extracts and filtrates of the bacillus, differing so strikingly from the acute illness with rapid death or equally rapid recovery resulting from anaphylatoxin poisoning, would justify the assumption that poisons of this nature may still play a part in the disease, adding an additional specific characteristic to the clinical picture. As stated before, however, it is not improbable that all these characteristics may represent merely a more protracted or subacute state of anaphylatoxin toxemia.

The experiments with autolysates, although none of them were fatal in their results upon guinea pigs, have sufficiently indicated that poisons comparable to anaphylatoxins can be formed in this way. This would indicate that a reaction of proteolysis, which may take place slowly by autolysis, is hastened by the action of complement, and its velocity is still further augmented by the increase, within certain limits, of the sensitization,—a conception which would attribute to the combined action of complement and sensitizer a function not incomparable to that of the bodies spoken of as catalytic agents.

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ORGANIC MATTER IN THE EXPIRED BREATH WITH ESPECIAL REFERENCE TO ITS INHIBITING POWER ON OXIDIZING FERMENTS.*

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In 1895 Billings, Mitchell, and Bergey (1) published the results of a large number of experiments showing that specific poisons of an organic nature are not present in expired air. These results reversed the generally prevailing opinion of sanitarians, who now began to account for the ill effects of overcrowding by physical agents rather than by specific poisons in the exhaled breath. Recently Weichardt (2) published the results of experiments which he claims prove the presence of poisons of an organic nature in the expired breath. His experiments on the effect upon mice of products collected from the expired breath of elderly persons have been criticized by Inaba (3) who believes Weichardt's results to be due rather to the heterotonic nature of the fluids injected than to toxins or protein poisons.

Weichardt also believes that he has detected the presence of split proteins by experiments *in vitro*. The vapor from the expired breath of a fatigued man sixty-three years old was filtered through cotton, passed through ten cubic centimeters of distilled water, and then concentrated *in vacuo* to three cubic centimeters. The solution containing the products of the expired breath left a residue weighing nine milligrams. This residue turned brown when heated on platinum foil and gave off odoriferous fumes. Weichardt also noted that a characteristic odor was produced when the solution of the respiratory products was acidified with hydrochloric acid or neutralized with sodium hydroxide.

Further evidence of the presence of organic matter in the expired breath is presented by Weichardt who found that the oxidation of guaiacum by blood is inhibited by the substances in the expired

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breath, just as it is prevented by diphtheria toxin. In the experiment designed to demonstrate this fact, one cubic centimeter of the fluid, containing the products from the expired air, was placed in each of a series of tubes. To each tube was then added a convenient amount of a very dilute solution of fresh blood, 0.1 of a cubic centimeter of the guaiacum being used as indicator.

Weichardt claims that this substance in expired air, which is capable of inhibiting the oxidation of guaiacum by blood, may also be found in the air of a closed room in which several persons have slept. He exposed 0.25 of a gram of finely powdered calcium chloride in a platinum dish covered with filter paper to keep out gross particles. On the following morning the calcium chloride was dissolved in water and the protein split products were demonstrated in the solution by means of the inhibition of the guaiacum reaction.

It is interesting to note that Weichardt claims to have obtained the toxic substances by allowing the expired breath to pass through normal hydrochloric acid, while Merkel (4) claims to have found a base which is poisonous when free, but innocuous after contact with an acid.

Rosenau and Amoss[1] (5) filtered the expired breath through two glass bulbs firmly packed with glass-wool and collected the moisture by condensing it in a Drechsel bottle set in a freezing mixture. This liquid when injected into guinea pigs exhibited no toxic effects, but sensitized the animals so that a subsequent injection of normal human serum produced the characteristic symptoms of anaphylaxis. By this method they have demonstrated the presence of a protein-like substance in the expired breath. This substance may or may not be toxic. This work has recently been corroborated by Jordan and Hixson,[2] who also obtained similar results with filtered saliva as the sensitizing agent. In view of the fact that filtered saliva sensitizes animals against a subsequent injection of normal blood serum, it may be argued that the experiments of Rosenau and Amoss are inconclusive.

[1] A brief review of the literature on the organic matter in the expired breath is given by Rosenau and Amoss (5). A complete digest of the work on the composition of expired air and its effect on animal life is given by Billings, Mitchell, and Bergey (1).

[2] Personal communication from Dr. E. O. Jordan.

These observers, however, carefully guarded against the contamination of the condensed liquid by saliva, and are certain that no contamination occurred. The liquid condensed was uniformly sterile. If any saliva solution had passed the two glass-wool filters, it is almost certain that this liquid would not have been free from bacteria.

The method employed in the following experiments for collecting the products of the expired breath was essentially the same as that used in the experiments of Rosenau and Amoss (5).

EXPERIMENTS WITH THE GUAIACUM REACTION.

It is well known that a 5 per cent. solution of gum guaiacum in alcohol takes on a blue color in the presence of blood and a small amount of oxygenated turpentine or hydrogen peroxide, that the delicacy of this test is variable, and that different samples of the commercial gum do not react with equal sensitiveness. Various modifications of the reagent have been proposed in order to make the tests more uniform and to increase the delicacy of the reaction as a test for blood. Among these modifications we find the method of Weichardt who purifies the resin by precipitating it from a solution in equal parts of acetic acid and alcohol by the addition of water. A purified product, called guaiacin, can be obtained on the market. A 5 per cent. emulsion of guaiacin in 70 per cent. alcohol was used in the following experiments.

Experiment 1.—The concentrated and dialyzed liquid condensed during five hours from the expired breath of S., plus dilute blood solution, plus two drops of ozonized turpentine, plus guaiacin indicator, gave a very slight blue color. Five c.c. of a weak solution of calcium chloride plus blood solution with ozonized turpentine gave a strong blue color with guaiacin. With the guaiacin indicator, a control of 5 c.c. of water and dilute blood solution showed about the same amount of blue color as the liquid condensed from the expired breath.

Experiment 2.—Pure calcium chloride was exposed in Petri dishes in a cabinet of 500 cubic feet capacity in which twelve persons remained for one hour. The persons came out quickly and the room was closed for the next twenty-four hours, during which time the five Petri dishes remained exposed to the vitiated air. The calcium chloride absorbed the moisture and the saturated solution of the salt thus obtained was added to dilute blood solution and tested with the guaiacin indicator. All tubes gave a blue color. This experiment was repeated with the same results. These results do not confirm those of Weichardt who found that calcium chloride in a Petri dish covered with a filter to keep

out particulate matter and exposed in a bedroom over night absorbed from the expired breath of the person sleeping there a substance which prevented the bluing of the guaiacin indicator by blood.

Experiment 3.—Calcium chloride was placed in sterile Petri dishes and breathed upon directly until a saturated solution of calcium chloride was obtained (two hours). A mixture of equal parts of this solution and blood solution were tested with the guaiacin, a blue color resulting immediately.

Experiment 4.—In order to determine the effect upon the guaiacin indicator of calcium chloride alone the following tubes were tested. The results are given below.

Solutions of calcium chloride.	Result of guaiacum.
1 per cent. solution	Slight bluing.
25 per cent. solution	Blue immediately.
1 per cent. solution made alkaline with sodium hydroxide	Yellow.
25 per cent. solution made alkaline with sodium hydroxide	Yellow.
1 per cent. solution made acid by acetic acid	Tan.
25 per cent. solution made acid by acetic acid	Tan.

These results are in accordance with those obtained by Alsberg (6), who found that various chlorides, with and without the aid of hydrogen peroxide, could bring about the bluing of guaiacum. Among the metals tried were cobalt, nickel, aluminum, copper, and iron; even sodium and potassium chlorides gave a positive test in the presence of hydrogen peroxide. "In all cases the reaction was most marked in neutral or weakly acid solution. Noticeable amounts of alkali or mineral acids retarded the reaction."[3] Calcium salts were not tested.

Smith and MacDonald (7) have shown that guaiacum gives a positive reaction with each of the mineral constituents of human saliva. It would seem, therefore, from these facts that the guaiacum test is unreliable. Alsberg, in fact, as the result of his experiments comes to the conclusion that the guaiacin reaction is extremely unreliable.[4] Are we therefore justified in assuming that an organic body exists in the expired breath when we obtain a negative test for blood with guaiacum in a solution known to contain blood and to which there has been added the fluid obtained in Weichardt's experiment? To answer this question a series of three tubes was prepared.

Experiment 5.—Tube 1 contained 5 c.c. of dilute blood solution; tube 2, 5 c.c. of dilute blood solution plus 2 drops of N/10 sodium hydroxide, and tube 3,

[3] Alsberg, C. L., *loc. cit.*, p. 43.

[4] Alsberg, C. L., *loc. cit.*, p. 52.

5 c.c. of blood solution plus 2 drops of N/10 hydrochloric acid. On testing these tubes with guaiacin indicator and oxygenated turpentine the following results were obtained:

Tube 1	Blue immediately.
Tube 2	Yellow,—later green.
Tube 3	Very light blue.

This inhibiting effect of small amounts of acids and alkalis on the guaiacum reaction for blood is more sharply and definitely shown by using 2.5 cubic centimeters of a 25 per cent. solution of calcium chloride with an equal amount of dilute blood solution. Thus with tubes 1, 2, and 3 (neutral, alkaline, and acid, as indicated above), the following results were obtained with the guaiacin indicator after three hours:

Tube 1	Deep blue.
Tube 2	Yellow.
Tube 3	Tan.

If sodium chloride was used instead of calcium chloride, the results were slightly different. For example, when the blood was diluted with physiological normal salt solution, the following results were obtained:

Tube 1	Deep blue.
Tube 2	Blue after 3 hrs.
Tube 3	Brown after 3 hrs.

As the result of the above experiments it is plain that factors other than some specific organic compound in the expired breath are able to account for the inhibition of the guaiacum test for blood as reported by Weichardt. It should be noted that this observer collected some of his material by allowing the expired breath to pass through normal hydrochloric acid and then concentrating and neutralizing the resulting solution. From these experiments it will be seen that if he did not add sufficient alkali for neutralization, or if he added too much, a negative result would be obtained with the guaiacum test.

RESIDUE ON EVAPORATION.

The expired breath of S. (May 5) was allowed to bubble through N/10 hydrochloric acid for five hours. The resulting liquid, amounting to 55 c.c., was evaporated to dryness on a water bath in a weighed platinum dish leaving a

residue of 0.0021 gm. On heating, dense white fumes were given off. There was no odor, blackening, or charring, and no weighable residue.

Experiment 6.—In a similar experiment, lasting four hours (S.), only 0.0004 gm. of residue was found in the 25 c.c. of liquid collected, but in this experiment the delivery tube inside the Drechsel bottle did not dip into the acid solution until some condensation had taken place. This residue was volatile at dull red heat.

Experiment 7.—Still another experiment of this kind was carried out under the same conditions as the first, with the result that in the 35 c.c. of liquid, 0.0019 gm. of residue was found and this was entirely volatilized with dense fumes. There was no odor, charring, or blackening.

Experiment 8.—Another experiment of four hours' duration, with the same person, resulted in the collection of 30 c.c. of liquid. This was evaporated to dryness on a water bath leaving a residue weighing 0.0014 gm. This residue was taken up in ammonia-free water and Nesslerized. Calculated as ammonium chloride, 0.001365 gm. was found. In each case there was noticed the characteristic odor mentioned by Weichardt, but his observations on the blackening by ignition were not corroborated.

The results in these experiments seem to indicate that the organic matter in the expired breath retained by hydrochloric acid consists, within the limits of experimental error, of ammonia. Lehmann and Jessen (8) found that more ammonia was present in the fluid collected from the breath of a person with decayed teeth than in that obtained from a person whose teeth were sound. Bergey (1) also found variable amounts of ammonia in the condensations of the expired breath.

THE EFFECT OF THE PRODUCTS OF THE EXPIRED BREATH ON THE OXIDATION OF PHENOLPHTHALIN BY BLOOD.

From the experiments already cited, the guaiacum test for blood seems so unreliable and so readily affected by many agents that no satisfactory conclusions can be drawn from the results of the inhibition of this test by products of the expired breath. The use of the phenolphthalin test for blood naturally suggests itself as a means of testing the correctness of Weichardt's findings. This reagent was first proposed as a test for plant oxidases by Kastle (9) and was later employed by Kastle and Amoss (10) to determine the variations in the oxidase and peroxidase activity of human blood in health and disease. This test depends upon the fact that phenolphthalin, which is the leuco-compound of phenolphthalein, is

colorless in alkaline solution and is readily oxidized to phenolphthalein, which in alkaline solution exhibits a characteristic deep purplish red color. This reaction is quantitative and is very delicate. Kastle and Amoss made quantitative determinations using blood in a dilution of one part in 25,000, and by employing this reagent Kastle (11) was able to detect one part of blood in 80,000,000 parts of water. By this method, moreover, the processes of oxidation by blood can be studied quantitatively and even the variations in the rate of reaction can be investigated.

THE PHENOLPHTHALIN REAGENT.

The oxidase reagent consists of 0.032 of a gram of phenolphthalin plus twenty-one cubic centimeters of N/10 sodium hydroxide made up to 100 cubic centimeters with water.

The peroxidase reagent consists of the same substances as the oxidase reagent plus ten cubic centimeters of 0.034 per cent. hydrogen peroxide, the whole being made up to 100 cubic centimeters with distilled water.

THE BLOOD SOLUTIONS.

For the oxidase tests, 10 c. mm. of blood of A., taken with a Zeiss red blood corpuscle pipette, were diluted to 100 c.c. with distilled water, making a dilution of 1:10,000. Two c.c. of the fresh blood solution were measured in a pipette, by means of the apparatus designed by Rosenau (12), into a glass stoppered bottle. There were then added 5 c.c. of the solution to be tested for the inhibitive action plus 5 c.c. of the oxidase reagent, all in a glass stoppered bottle which was then set aside for twenty-four hours at room temperature. The peroxidase tests were made in the same manner except that the 10 c. mm. of blood were diluted to 250 c.c., making a dilution of 1:25,000. For the sake of convenience and for comparison of the oxidase and peroxidase tests, the oxidation was allowed to proceed at room temperature for about twenty-four hours. As a matter of fact, the percentage of oxidation in the peroxidase tests is apparently greater at the end of one hour than at the end of twenty-four hours, due to the fading in color of the phenolphthalein in alkali, to the presence of hydrogen peroxide, and to another influence, for Kastle and Amoss (10) found the fading to vary with the character of the blood, "being more rapid in the case of diseased bloods, poor in hemoglobin." The greatest depth of color in the oxidase tests was found after twenty-four hours.

The percentage of oxidation in each case was determined by comparing the colored solution with a known solution of phenolphthalein in alkali by means of a Duboscq colorimeter. This standard solution differed slightly from the solution usually employed, being made up of 0.0318 gm. of phenolphthalein in 50 per

cent. alcohol (dissolved first in 50 c.c. of absolute alcohol and then diluted with water to 100 c.c.), and 1 c.c. of this solution plus 1 c.c. of N/10 alkali plus 10 c.c. of water, making a total dilution of 12 c.c. to correspond with the total volume of the tests.

It was found that a permanent standard could be made with a dilute aqueous solution of basic fuchsin and a very small amount of Loeffler's methylene-blue. In a glass stoppered bottle this standard keeps well and without change for a month. It is evident that such a standard saves the time necessary to measure out carefully the phenolphthalein solution and the N/10 alkali.

The standard cell in the colorimeter is set at a convenient number, generally at 5 or 10 mm. It will be seen by calculating the amounts of phenolphthalin oxidized and of phenolphthalein formed, and the percentage in each case, that a simple formula can be obtained whereby the end results may be secured by a single calculation. If x is the reading, and the standard set is at 10 mm., then 100/x is the percentage of phenolphthalin oxidized. If the standard is set at 5, then 200/x is the percentage of phenolphthalin oxidized.

Experiment 1.—The vapor from the expired breath of M. (female, age 23 years) was condensed for a period of three hours in the Drechsel bottle already described, and set in a freezing mixture. The total amount was 20 c.c. (solution 1).

The vapor in the expired breath of L. (male, age 26 years) was also collected in the same manner in a separate apparatus for a period of two hours. The total volume was 15 c.c. (solution 2). The expired breath of R. (male, age 19 years) was passed for three hours through 10 c.c. of N/10 hydrogen chloride, using a 16 oz. Drechsel bottle set in water at 20° C. This solution was neutralized with N/10 sodium hydroxide (solution 3). Its total volume was 30 c.c. The solutions were brought to room temperature and tested for inhibiting power on the peroxidase reaction of the diluted blood of L. (1:25,000) in the manner already described. At the end of one hour, and of thirty hours, they were compared in the tintometer against the standard set at 5 mm., with the following results:

Solution No.	Reading after 1 hr.	Per cent. of phenolphthalin oxidized.	Reading after 30 hrs.	Per cent. oxidized.
1	3.2	31.2	3.2	31.2
2	3.0	33.3	3.0	33.3
3	3.0	33.3	3.0	33.3
Control	2.9	34.5	2.9	34.5

These figures show slightly less oxidation in the case of the condensed liquids than in the control. All, however, agree within the limits of experimental error.

Experiment 2.—Vapor from the expired breath of M. was collected for three hours in an apparatus placed in a freezing mixture (solution 1). The solution was kept cold until used (twenty-four hours). The vapor from the expired breath of M. was collected for two hours by passing it through a Drechsel bottle containing 10 c.c. of N/10 hydrochloric acid. During the collection, the

bottle was kept in a freezing mixture. The fluid was concentrated *in vacuo* to one third its volume and 10 c.c. of N/10 sodium hydroxide were added (solution 2). The solution was kept in a cold room until used (twenty-two hours). As in solution 2, vapor from the expired breath of M. was passed through N/10 hydrochloric acid in the cold. The acid was neutralized and the fluid was then placed in a tube for concentrating colloids by means of a direct current. A direct current of 110 volts and about 0.1 ampere was passed through this solution for three hours. The liquid in the cathodal end of the tube was marked solution 3.

These solutions were tested in duplicate for any inhibitory effects on the oxidation by peroxidases and oxidases, and the readings were compared at the end of twenty-four hours. The standard was set at 10 mm.

Solution No.	Peroxidase test.		Oxidase test.	
	Reading on tintometer when compared with standard set at 10 mm.	Per cent. of phenolphthalin oxidized.	Reading on tintometer when compared with standard set at 10 mm.	Per cent. of phenolphthalin oxidized
1	6.3	31.5	3.1	64.5
1	6.3	31.5	3.1	64.5
2	6.0	33.3	2.4	83.3
2	6.0	33.3	2.4	83.3
3	6.4	31.2	4.1	48.8
3	6.4	31.2	4.1	48.8

The results in this experiment show practically no difference in the amount of oxidation in the case of the peroxidases. However, in the oxidase test the simple condensations in the cold (solution 1) showed some inhibiting power (20 per cent.) over the acid collections (solution 2), and the greatest diminution was in the case of the concentrated solution. It was thought that if organic matter were in the expired breath in the form of colloids, they would travel in the direction of the current and collect in the cathodal end of the tube.[5] Of course the presence of sodium chloride in the solution should be considered as the first possible cause of any inhibition of the oxidase reaction. The ends of the tubes were closed by celloidin membranes, but there was hardly sufficient area to allow complete dialysis. In fact, chlorides were found in the liquid after the passage of the current was discontinued.

Experiment 3.—Another attempt at concentration by the direct current was made on March 18. For two hours on three successive days M. breathed into

[5] Dr. C. S. Hudson in a personal communication states that he has been able to concentrate invertase by this method even to the extent of obtaining this ferment free from nitrogen.

10 c.c. of N/10 hydrochloric acid in the same apparatus, placed in water at 20° C. At the end of each period the liquid was neutralized with N/10 sodium hydroxide and immediately placed in the concentrating tube. The current was allowed to flow over night. When new liquid was added each day, that in the cathodal end was kept and the remainder replaced by new material. It was planned to concentrate in this manner the expired breath for six hours, allowing the minimum time for any possible decomposition. The fully concentrated liquid was dialyzed for two hours in a collodion sac (solution 1). The liquid in the anodal end of the tube constituted solution 2.

Five gm. of calcium chloride were exposed for forty-eight hours to the air of a closed cabinet of 300 cubic feet capacity in which eight persons had been for one hour. The carbon dioxide content went up to 6.5 per cent. The calcium chloride absorbed water to about 15 c.c. of the total volume, and the solution thus formed was dialyzed in a celloidin sac for two hours (solution 3). Five gm. of calcium chloride were dissolved in 15 c.c. of water (solution 3 B).

Together with the solutions described above a control of distilled water was tested for its effect on the peroxidase and oxidase reactions, and gave the following results:

Solution No.	Peroxidase test.		Oxidase test.	
	Reading on tintometer when compared with standard set at 10 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.	Reading on tintometer when compared with standard set at 10 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.
1	6.1	32.8	18.4	10.9
1	6.2	32.3	18.4	10.9
2	15.0	13.3	33.0[6]	6.6
2	15.5	13.0	33.0[6]	6.6
3	8.1	24.7	60.0[7]	3.5
3	8.1	24.7	60.0[7]	3.5
3 B	12.3	16.3	Very faint pink	—
3 B	12.3	16.3		—
Control	6.2	32.3	2.5	80.0
Control	6.2	32.3	2.5	80.0

In this series we find that the concentrated products of the expired breath of one person during a period of six hours (solution 1) had no effect on the peroxidase reaction, but the oxidase reaction was greatly inhibited. Strangely enough the liquid left over from such a

[6] In this case the standard was set at 3 mm. and the reading was 9.9. When this is calculated in terms of the standard set at 10 mm., we get 33.

[7] Estimated, but too faint for exact comparison.

concentration (solution 2) showed decided inhibition in the peroxidase tests and a greater inhibitory action on the oxidase test than the concentrated liquid. The calcium chloride that had been exposed in the cabinet, and the control showed marked effect on the peroxidase and the oxidase. It is to be noted that the control (solution 3 B) was not dialyzed.

The results in this series direct our attention to the effect of the salts present on the reactions. The mixtures were accordingly titrated by Volhard's method with N/10 silver nitrate.

Solution.	Salt present.	N/10 silver nitrate required in c.c.	Amount of salt present in gm.
Control	None	0.0	———
1	NaCl	0.6	0.0029 NaCl.
2	NaCl	1.4	0.0055 NaCl.
3	$CaCl_2$	47.0	0.2608 $CaCl_2$.
3 B	$CaCl_2$	48.0	0.2664 $CaCl_2$.

These results show that the inhibiting effect is roughly proportional to the amount of inorganic matter present. In order to determine the effects of the salts which might be found in the various fluids in the attempt to absorb any organic matter that might be present in the expired breath, a series of experiments with sodium chloride, calcium chloride, and ammonium chloride were set up in both the peroxidase and the oxidase tests.

Experiment 4.—Two c.c. of blood were added to varying amounts of the salts and water to make a series of concentrations, in each case with a total volume of 12 c.c. from N/5 to N/100.

Amount of N/1 taken.	Amount of water added.	Solution No.
2.4 c.c. to make 12 c.c. of N/5	2.6 c.c.	1
1.2 c.c. to make 12 c.c. of N/10	3.8 c.c.	2
0.6 c.c. to make 12 c.c. of N/20	4.4 c.c.	3
0.24 c.c. to make 12 c.c. of N/50	4.76 c.c.	4
0.12 c.c. to make 12 c.c. of N/100	4.88 c.c.	5

These tests were allowed to stand at room temperature for twenty-four hours and were then compared with the standard with the following results:

Solution containing blood plus reagent.	Peroxidase reaction.		Oxidase reaction.		N/10 HCl in c.c. required to discharge pink color.
	Reading on tintometer when compared with standard set at 5 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.	Reading on tintometer when compared with standard set at 5 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.	
N/5 NaCl.....	6.6	15.1	2.0	50.0	0.9
N/5 NaCl.....	6.6	15.1	2.0	50.0	0.9
N/10 NaCl.....	6.5	15.4	1.7	58.8	0.9
N/10 NaCl.....	6.5	15.4	1.7	58.8	0.9
N/20 NaCl.....	6.6	15.1	1.8	55.5	0.9
N/20 NaCl.....	6.6	15.1	1.8	55.5	0.9
N/50 NaCl.....	4.2	23.8	1.8	55.5	0.9
N/50 NaCl.....	6.5	15.4	1.8	55.5	0.9
N/100 NaCl.....	4.2	23.8	1.8	55.5	0.9
N/100 NaCl.....	6.5	15.4	1.8	55.5	0.9
N/5 $CaCl_2$.....	10.6	9.5	40.0	2.5	0.9
N/5 $CaCl_2$.....	10.6	9.5	40.0	2.5	0.9
N/10 $CaCl_2$.....	8.0	12.5	30.0	3.3	0.9
N/10 $CaCl_2$.....	8.0	12.5	30.0	3.3	0.9
N/20 $CaCl_2$.....	6.2	16.0	18.0	5.5	0.9
N/20 $CaCl_2$.....	6.2	16.0	18.0	5.5	0.9
N/50 $CaCl_2$.....	6.0	16.7	3.2	31.2	0.9
N/50 $CaCl_2$.....	6.0	16.7	3.2	31.2	0.9
N/100 $CaCl_2$.....	5.5	18.1	5.0	20.0	0.9
N/100 $CaCl_2$.....	6.0	16.7	5.0	20.0	0.9
Control.....	6.8	14.7	1.6	62.5	0.9
Control.....	7.5	13.3	1.6	62.5	0.9

Experiment 5.—In testing the effect of sodium and ammonium chlorides the following results were obtained after twenty-four hours:

Solution containing blood plus reagent.	Peroxidase reaction.		Oxidase reaction.	
	Reading on tintometer when compared with standard set at 5 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.	Reading on tintometer when compared with standard set at 5 mm. Average of 5 readings.	Per cent. of phenolphthalin oxidized.
0.85% NaCl.....	6.4	15.6	7.6	12.0
0.85% NaCl.....	8.5	18.1	8.0	12.5
N/5 NaCl.....	5.5	18.1	5.0	20.0
N/5 NaCl.....	4.8	21.2	6.0	16.7
N/10 NaCl.....	4.5	22.2	5.0	20.0
N/10 NaCl.....	4.5	22.2	5.0	20.0
N/20 NaCl.....	5.0	20.0	5.0	20.0
N/20 NaCl.....	5.5	18.1	5.0	20.0
N/5 NH_4Cl.....	26.0	3.8	Very slight	——
N/5 NH_4Cl.....	30.0	3.3	pink.[8]	——
N/10 NH_4Cl.....	10.0	10.0	29.0	3.4
N/10 NH_4Cl.....	12.0	8.3	29.0	3.4
N/20 NH_4Cl.....	5.5	18.1	8.2	12.2
N/20 NH_4Cl.....	5.5	18.1	8.2	12.2
N/50 NH_4Cl.....	5.5	18.1	5.5	18.1
N/50 NH_4Cl.....	5.5	18.1	5.5	18.1
N/100 NH_4Cl.....	5.0	20.0	5.5	18.1
N/100 NH_4Cl.....	5.0	20.0	5.5	18.1
Control.....	4.8	21.2	5.2	19.2
Control.....	5.0	20.0	5.0	20.0

Sodium chloride solutions up to N/5 seem to have no effect on the blood peroxidase and oxidase reactions with phenolphthalin in alkali. When the whole solution is made isotonic there is slight diminution in the peroxidase activity and more in the oxidase reaction. This is in harmony with the fact recently brought out that the catalase activity of the blood is decreased by preventing the laking of blood. Calcium chloride and ammonium chloride in higher concentrations show an inhibiting power on the peroxidase reaction. Calcium chloride in N/100 solution affects the oxidase reaction, and this effect is increased with the concentration of the calcium. That the diminution was not due to any acidity of the salts is seen from the fact that the amount of N/10 hydrochloric acid necessary to

[8] Deepened slightly on addition of more alkali.

discharge the color in every case was the same. Ammonium chloride in N/20 solution also decreases the oxidase reaction as the concentration of the salt increases; in N/5 solution there is very little oxidation. It is to be noted that in the latter case there was a slight deepening of the color on the addition of more alkali, showing that there was some oxidation. This oxidation, though slight, was hidden, probably on account of the decrease in alkalinity due to a loss of ammonia.

The results are such as one would expect. Theoretically the stronger base and acid would unite leaving the weaker base to form a salt with the phenolphthalin. In the calcium chloride experiment theoretically we should have the sodium of the phenolphthalin salt in the reagent and any excess as a free base unite with the chlorine of the calcium chloride leaving the calcium to form the organic salt and to furnish the alkalinity. Ammonium chloride acts in this way, so our experiment shows that the calcium and ammonium salts of phenolphthalin do not serve well as indicators for the peroxidases and especially the oxidases.

Experiment 6.—Since guaiacum has been shown to give a positive reaction with certain salts it was decided to test the conduct of the phenolphthalin reagent towards the salts alone. To that end 2 c.c. of water were used instead of dilute blood solution. The solutions therefore contained 5 c.c. of the reagent, 7 c.c. of water, and sufficient salt to make the total solution N/5.

At the end of twenty-four hours the tests showed the following results:

Peroxidase reagent.	Oxidase reagent.
Control—faint pink.	Control—very faint pink.
N/5 calcium chloride—very faint pink.	N/5 calcium chloride—colorless.[9]
N/5 sodium chloride—3.3 per cent. oxidation.	N/5 sodium chloride—5.5 per cent. oxidation.

Sodium chloride in N/5 solution seems to effect a slight oxidation of phenolphthalin in twenty-four hours with and without the aid of hydrogen peroxide. Kastle reports a faint pink color in a similar experiment.

THE EFFECT OF SALTS ON THE COLOR OF PHENOLPHTHALEIN IN ALKALI

Experiment 7.—It is to be expected that the presence of salts of a weak base should decrease the depth of color of phenolphthalein in alkali. Accordingly

[9] The addition of alkali gave no color.

1 c.c. of phenolphthalein standard plus 7.6 c.c. of water plus 1 c.c. of N/10 sodium hydroxide plus 2.4 c.c. of N/1 ammonium chloride solution (making a total of 12 c.c. of N/5 ammonium chloride solution) was compared with 1 c.c. of standard phenolphthalein solution plus 10 c.c. of water plus 1 c.c. of N/10 sodium hydroxide. The result was that a layer of 5 mm. of the solution without ammonium chloride was equal in intensity to a layer 22 mm. thick of the solution plus ammonium chloride. In other words, the presence of ammonium chloride to the extent of N/5 solution decreases the color of the standard phenolphthalein reagent by 80 per cent.

FURTHER EXPERIMENTS WITH EXPIRED AIR.

Experiment 7.—For three hours a day for six successive days the expired breath of M. was blown through the same sterile Drechsel bottle in which there were 10 c.c. of N/10 hydrochloric acid. The bottle was set in water at 20° C. and was not placed in the cold during the intervals between the blowing periods. The two glass-wool filters were kept warm by the heat from an electric light and the first filter was replaced by a dry sterile filter when there was visible condensation or contamination with saliva.

At the end of the last breathing period 11 c.c. of N/10 sodium hydroxide were added making the total volume 75 c.c. (solution 1). This liquid reacted slightly alkaline to litmus. Some of solution 1 was placed in an Arnold sterilizer (temperature 100° C.) for twenty minutes, taken out, and allowed to come to room temperature (solution 2).

With a similar apparatus and with the same technique that was used for solution 1, the expired breath of R. for twelve hours, of M. for two hours, and of L. for seven hours, all in the same apparatus, was passed through 10 c.c. of N/10 hydrochloric acid. This solution was kept at 44° F. for six hours before it was brought to room temperature and 11 c.c. of sodium hydroxide were added (solution 3). The total volume was 90 c.c. Some of solution 3 was heated in an Arnold sterilizer at 100° C. for twenty minutes and was then allowed to cool (solution 4).

Ten c.c. of N/10 hydrochloric acid plus 55 c.c. of redistilled water were placed in a sterile Drechsel bottle, and carbon dioxide from a cylinder of the compressed gas was allowed to bubble through for five minutes. Eleven c.c. of N/10 sodium hydroxide were added (solution 5). Some of solution 5 was heated in an Arnold sterilizer (solution 6).

The control consisted of 10 c.c. of N/10 hydrochloric acid plus 11 c.c. of N/10 sodium hydrate.

These solutions were tested for their effect on the peroxidase and oxidase reactions in the usual manner excepting that the blood solutions were in contact with the various solutions to be tested for thirty minutes before the reagents were added. At the end of twenty hours the following results were obtained:

Solution No.	Peroxidase reaction.		Oxidase reaction.	
	Reading of tintometer when compared with standard set at 5 mm.	Per cent. of phenolphthalin oxidized.	Reading of tintometer when compared with standard set at 5 mm.	Per cent. of phenolphthalin oxidized.
1	6.1	16.4	1.4	71.4
1	6.1	16.4	1.4	71.4
2	8.0	12.5	2.5	40.0
2	5.8	17.2	2.5	40.0
3	7.5	13.3	1.4	71.4
3	6.1	16.4	1.4	71.4
4	5.9	16.9	1.9	52.6
4	5.9	16.9	1.9	52.6
5	No color	0	No color	0
5	No color	0	No color	0
6	5.8	17.2	2.8	15.7
6	5.8	17.2	2.8	15.7
Control	5.5	18.1	1.9	52.6
Control	5.5	18.1	1.9	52.6

These results show that practically no effect is produced on the peroxidase reaction by the products of expired breath retained by hydrochloric acid. The effect of an excess of carbon dioxide in preventing the reaction absolutely is what might be expected for the reason that carbon dioxide reacts more strongly acid than the phenolphthalin. The carbon dioxide, therefore, combines with the sodium hydroxide and prevents the formation of the sodium salt of the phenolphthalin so that the oxidation to phenolphthalein is not accomplished. The results in the oxidase test show some interesting variations. It cannot be said now what factor is able to influence the process as shown in solutions 1 and 3 and solutions 2 and 4. The higher percentages over the control in solutions 1 and 3 are perhaps due to the slight excess of alkalinity, as an excess of one cubic centimeter of N/10 sodium hydroxide was added, in one case accidentally, and in the other cases to make the tests uniform. It will be remembered that the volumes in solutions 1 and 3 were less, and the alkalinity for a given quantity would, therefore, be increased. The decrease after heating is not understood, but it may be due to the decrease in dissolved oxygen in the solution. In the case of solution 6, the acid carbonate remaining after drawing off

the carbon dioxide did not favor the reaction in the same manner that the carbonate does.

Experiment 8.—In still another experiment the expired breath was forced through 10 c.c. of N/10 hydrochloric acid for nineteen hours (S., seven hours, R., four hours, and L., eight hours) in the manner already described. The liquid was kept in the cold for five hours before testing. Ten c.c. of N/10 sodium hydroxide were added after the solution had been brought to room temperature (solution 1). The total volume was 90 c.c.

One c.c. of saliva was diluted to 80 c.c. with distilled water, 10 c.c. of hydrochloric acid were added and the whole solution was well shaken. After the solution had stood for thirty minutes, 10 c.c. of N/10 sodium hydroxide were added (solution 2). The control consisted of 10 c.c. of N/10 hydrochloric acid plus 70 c.c. of distilled water plus 10 c.c. of N/10 sodium hydroxide.

After thirty hours the following results were obtained:

Solution No.	Peroxidase reaction.		Oxidase reaction.	
	Reading of tintometer when compared with standard set at 5 mm.	Per cent. of phenolphthalin oxidized.	Reading of tintometer when compared with standard set at 5 mm	Per cent. of phenolphthalin oxidized.
1	5.8	17.2	1.5	66.6
1	5.8	17.2	1.5	66.6
2	6.5	15.4	1.5	66.6
2	3.5	28.6	1.5	66.6
Control	3.5	28.6	1.5	66.6
Control	3.5	28.6	1.5	66.6

In spite of careful manipulations the duplicate in the peroxidase series shows a variation of over 10 per cent. However, we have proved that a contamination of one cubic centimeter of saliva (more than could possibly have gotten through the filters) shows no effect on the oxidase reaction. Using the same apparatus for the same length of time, Rosenau and Amoss (5) found no bacterial contamination where care was exercised in the breathing experiments. The test with solution 1 shows that we were unable to obtain an inhibitory agent by continuously passing the expired breath through hydrochloric acid for a period of nineteen hours.

SUMMARY AND CONCLUSIONS.

Weichardt claims to have demonstrated the presence of an organic body in the expired air. He allowed the exhaled breath to pass through hydrochloric acid solution, evaporated the resulting

solution to dryness on a water bath, and obtained a weighable residue which charred on ignition. If he neutralized the acid solution and concentrated it under reduced pressure, he obtained a solution which inhibited the bluing of the guaiacum indicator by blood. By exposing calcium chloride in a room in which the air had been vitiated, he claimed also to have obtained a substance from the air which prevented the bluing of the guaiacum indicator by blood.

The experiments here recorded show that a variable amount of matter is retained by weak hydrochloric solution when exhaled breath is passed through it, and that this matter is volatile on ignition. Contrary to the findings of Weichardt, there is no charring or blackening. Nesslerization shows the residue to consist mainly, if not wholly, of ammonium chloride. This ammonia is believed to have come from the decomposition of food particles about the teeth. In one case the person (S.) had smoked just before the experiment, so that a small amount of the ammonia from the tobacco smoke may have been held temporarily by the saliva and food particles in the mouth and been given off gradually during the experiment. Weichardt's experiments on the inhibition of the guaiacum test for blood by means of the substances retained when exhaled breath is passed through hydrochloric acid or over calcium chloride crystals are not corroborated. It is further shown that the guaiacum indicator is unreliable for these tests in view of the fact that a small amount of free acid or free alkali will inhibit the guaiacum test for blood. This fact is offered as a probable explanation of Weichardt's results.

Calcium chloride alone gives a deep blue color with the guaiacin indicator. Weichardt used this salt to collect from the expired breath certain unknown substances which he claims inhibit the oxidation of guaiacum by blood. His results are therefore inconclusive.

The phenolphthalin test for blood has been studied in this connection and further light has been thrown on this reaction. The sodium salt of phenolphthalin is colorless in alkaline solution, and is readily oxidized by minute quantities of blood to phenolphthalein which gives a characteristic deep purplish red color in alkaline solution.

It has been found that the presence of calcium chloride and ammonium chloride in small amounts retards and, in large amounts, prevents this reaction. It is believed that any salt composed of a weak base combined with a strong acid will have the same effect. This is discussed in the text. It has also been shown that the presence of calcium chloride or ammonium chloride decreases the depth of color of phenolphthalein in sodium hydroxide solution.

Carbon dioxide also prevents the oxidation of phenolphthalin by blood. Of course this does not mean that carbon dioxide prevents the action of the oxidizing ferments generally. In this particular case the substance to be oxidized, namely phenolphthalin, was not allowed by reason of the presence of the carbon dioxide to combine with the alkali and thereby assume a state in which it could be easily oxidized.

The results of one experiment seem to indicate a relation between the amount of dissolved oxygen in the solutions and the percentage of oxidation. Sodium chloride either alone or with the aid of hydrogen peroxide is able to bring about the oxidation of phenolphthalin in alkali to a very slight extent (3.5 to 5 per cent. in twenty-four hours). Therefore phenolphthalin as a test for oxidizing ferments should not be used in the presence of an appreciable amount of inorganic salts or carbon dioxide. Complete dialysis is recommended in these cases. It is also to be noted that the great delicacy of the test allows considerable dilution.

Liquids were obtained from the expired breath by passing this through weak hydrochloric acid or by condensing the moisture in it by conducting it through cooled Drechsel bottles. Attempts were then made to prove the presence in these liquids of some substance which inhibits the oxidation of phenolphthalin by blood, but all were unsuccessful. Moreover attempts to concentrate these liquids by evaporation under reduced pressure or by the passage of a direct current (colloidal travel) were also unsuccessful.

It is planned to improve upon the apparatus used to concentrate colloids by the passage of a direct current, and to test the effect of expired breath products on the rate of oxidation of phenolphthalin by blood.

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A COMPARISON OF THE ACTION OF PLASMA AND SERUM ON CERTAIN OBJECTS USED IN BIOLOGICAL TESTS FOR EPINEPHRIN.*

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Plates 11 to 21.

O'Connor,[1] working with citrate plasma on the frog perfusion preparation of Laewen, came to the conclusion that this plasma exerts no constrictor effect on the blood vessels of the frog, while serum to which the same amount of citrate has been added possesses the constrictor action demonstrated by Battelli and others for various kinds of serum.[2] With hirudin plasma and serum applied to segments of intestine and uterus, O'Connor states that the same difference can be demonstrated as regards the tone-increasing effect, plasma being without or almost without action, while the defibrinated blood plus hirudin shows the stimulating action. Yet he admits that it is difficult to obtain faultless experiments on these two objects.

Trendelenburg,[3] working with hirudin material on the frog perfusion preparation, was convinced that the plasma has a constrictor effect which stands to that of the corresponding serum in a definite ratio (1:2½ to 3).

Kahn[4] states that he has confirmed on the frog perfusion preparation the result that hirudin plasma in most cases has a smaller constrictor effect than the corresponding serum. The effect of hirudin plasma, according to Kahn, is much less constant than that of the serum. He does not agree with Trendelenburg that any definite ratio exists between the amount of the effect produced by the plasma and by the serum.

There seems to be a tendency to generalize such results into the conclusion that serum exerts on smooth muscle in general a stimulating or tone-increasing action which is absent from, or feebly developed in plasma obtained in any way whatever. It is clear, however, that a general statement contrasting the action of plasma and serum on smooth muscle organs can be drawn only after a comparison (1) of sera with plasmas obtained in as many different ways

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[1] O'Connor, J. M., *Arch. f. exper. Path. u. Pharmakol.*, 1912, lxvii, 195.

[2] Battelli, F., *Jour. de physiol. et de path. gén.*, 1905, vii, 625, 651.

[3] Trendelenburg, P., *München. med. Wchnschr.*, 1911, lviii, 1919.

[4] Kahn, R. H., *Arch. f. d. ges. Physiol.*, 1912, cxliv, 251.

as possible from one and the same blood on one and the same test object, and (2) of each kind of plasma and the corresponding serum on as large a series of different test objects as possible. There is reason to believe that, within a wide range at any rate, the influence of differences in the species of the animal from which blood on the one hand, and the test objects on the other are obtained is not important.

We attempted a partial survey of this kind by investigating the action of three kinds of plasma, (a) citrate, (b) peptone, (c) hirudin, on four test objects, (a) artery rings, according to Meyer's method, (b) the frog perfusion preparation, (c) intestine, (d) uterus segments. In addition, observations were made on the same objects with certain transudates and exudates. The transudates, for example hydrocele fluid, were chiefly interesting from our point of view as affording the means of comparing serous liquids which certainly had not clotted and had no tendency to clot spontaneously, with serum. They are, for convenience, described in a separate paper,[5] but the results are also used in the discussion in this paper. Some results of interest in connection with the general subject were also obtained by comparing the effect on the different test objects of serum treated in various ways. This work, which is still in progress, will be alluded to briefly here and merely in so far as it bears directly upon the question discussed in this paper. The behavior of some of the test objects to certain foreign proteins was also studied from the same standpoint.

TECHNIQUE OF OBTAINING THE MATERIAL.

Citrate Material.—Citrate plasma was always obtained by the following procedure. A cannula and attached rubber tube were boiled, oiled, and filled with a citrate solution made by dissolving sodium citrate in Ringer to the extent of 2 per cent. After the cannula had been inserted into the carotid of a dog, the end of the rubber tube was dipped below the citrate solution and a volume of blood equal to the citrate-Ringer solution was run in. Then the blood and solution were stirred gently but sufficiently to insure proper mixing. In case the plasma and serum were to be used for perfusion experiments the blood was kept in the incubator for one to one and one-half hours.[6] Each fluid used for perfusion was also filtered before injection. The serum for comparison was

[5] Stewart, G. N., and Zucker, T. F., *Jour. Exper. Med.*, 1913, xvii, 174.

[6] Trendelenburg, P., *Arch f. exper. Path. u. Pharmakol.*, 1910, lxiii, 161.

derived from defibrinated blood of the same animal to which an equal volume of citrate-Ringer solution was added while the mass of fibrin was still floating in the blood. After mixing the fibrin was removed. In a few experiments we used whole blood for the rings, the intestine, and the uterus preparation, but in the majority we used serum and plasma. For the perfusion experiments, it was of course necessary to centrifugalize repeatedly until all the corpuscles were separated. In addition to citrate serum, serum to which no citrate had been added, usually from the clotted blood, was also generally tested for the sake of comparison.

Peptone Plasma.—The technique for obtaining this is given in the protocols of the experiments quoted.

Hirudin Material.—The technique for obtaining hirudin plasma was practically the same as for citrate plasma. The blood was run into a solution containing 1 mg. of hirudin per cubic centimeter of Ringer solution. The proportion of hirudin solution used varied, and is given in the protocols of the separate experiments. The serum for comparison was obtained by diluting the defibrinated blood with the proper amount of hirudin solution before centrifugalization. The blood was kept in the incubator for a time before separating the plasma and serum if they were to be used for perfusion. In all the experiments except those with the material of September 26 the hirudin plasma was obtained from dogs. The material of September 26 was purposely obtained from rabbits in order to determine whether the use of homologous material made any difference as regards the intestine and uterus preparations.

TECHNIQUE OF MAKING THE TESTS.

Vessel Rings.—The test object consisted of a ring about 1.5 mm. wide cut from a sheep's carotid artery. With practice the width of the rings can be made nearly uniform. In a small cylindrical glass vessel with a capacity of about 2 c.c. the ring was held between two hooks, one fastened to the bottom of the vessel, the other connected with the lever by a light silk thread. The hooks were made of aluminium wire. After the ring was suspended in this way the vessel was filled with Ringer solution, lowered into the bath, kept between 39° and 40° C., and oxygen was bubbled through the solution by means of a side tube near the bottom of the vessel. In the present work the weight used in stretching was 10 gm. The time allowed for stretching was five minutes, this being found practically as effective in producing a sufficient permanent relaxation as a period of fifteen to twenty minutes. Stretching with a heavier weight did not, according to our experience, appreciably increase the sensitiveness of the rings to serum. After stretching, the weight was removed and the preparation allowed to come to equilibrium. Then the fluid to be tested was made to replace the Ringer solution by means of a bent pipette drawn out so as to reach to the bottom of the vessel without disturbing the ring. The ring was allowed to contract under the weight of an ordinary muscle lever, the shortening of the ring being magnified twenty-one times. In most cases when plasma was used which gave no effect or only a slight effect, this was replaced by an active serum. In this case the serum always gave a smaller effect than when used to displace the original Ringer solution. Therefore, for comparison only those curves were used which were obtained in the same way.

Perfusion.—In the frog perfusion experiments the technique used was essentially that of Trendelenburg.[7] At first considerable difficulty was experienced in inserting the cannulas. It was found later that this was largely due to the fact that the frogs had been freshly caught. Their vascular sensibility was so great that immediately on cutting the vessels contracted sufficiently to make it well nigh impossible to insert even the smallest cannula. When kept in a tank indoors without food they apparently became much less sensitive in this respect, and after a week or two the difficulty disappeared. Pithing the frog twelve to twenty-four hours before the experiment also rendered the insertion of the cannulas easier. Care must of course be exercised in putting on the ligatures so that leakage is avoided as far as possible. Still, with every care, some oozing of fluid is likely to occur and occasional drops may form and run down from the preparation without passing through the outflow cannula. Since, however, when the preparation is properly made the outflow from the cannula proceeds with great regularity and includes by far the greatest part of the perfused liquid, slight leakage does not seriously invalidate the experiment.

In the experiments in which citrate plasma was used the perfusion fluid contained 0.5 per cent. sodium citrate. But in order to allow clotting to occur as far as possible at the cut surfaces to close up the small vessels which could not be ligated, the perfusion was always begun with ordinary Ringer solution, two Mariotte bottles, one containing Ringer and the other citrate-Ringer, being connected by a T-piece with the perfusion system. It was found advantageous to avoid high pressure in perfusing because the higher the pressure the sooner excessive edema sets in, which diminishes considerably the rate of perfusion. The amount injected and the dilutions are given in the curves reproduced in figures 2 and 3 and in the descriptions of figures 4 and 5. We agree with the observation of Broeking and Trendelenburg[8] that "like all forms of biological testing, the perfusion method gives perfectly constant and satisfactory results only after long experience."

Intestine and Uterus Preparations.—The technique was the same as that previously employed by one of us.[9] The smaller apparatus, holding 2.5 c.c. without the preparation, was used throughout. Unless otherwise stated, enough liquid (2 c.c.) was always added to displace the whole of the liquid initially present.

RESULTS.

CITRATE PLASMA AND SERUM.

Experiments were made with eight samples of material from different animals.

Artery Rings.—The results were practically uniform. With the rings the citrate plasma usually gave a small effect. Where no effect was given the serum also had a relatively small action. Accordingly we recognized as suitable for our purpose only arteries

[7] Trendelenburg, P., *Arch. f. exper. Path. u. Pharmakol., loc. cit.*

[8] Broeking, E., and Trendelenburg, P., *Arch. f. klin. Med.*, 1911, ciii, 168.

[9] Stewart, G. N., *Jour. Exper. Med.*, 1911, xiv, 377; 1912, xv, 547.

which gave a decided effect with serum. The corresponding citrate serum invariably produced a greater effect than the plasma, and in all the experiments but one a much greater effect.

In reporting the artery ring experiments it would be without object to express the results in numbers,—for instance, the maximum rise obtained, or the latent period which elapsed before the rise began, or the amount of the rise which took place in a given time following the change of liquid,—since such numbers would give to our results by this method an appearance of quantitative accuracy which would be fallacious. The following designations will therefore be used. Slight rise, as in figure 1, observation 14; moderate rise, as in figure 1, observation 5; good rise, as in figure 13, observation 18; very good rise, as in figure 1, observation 16. In some cases it was doubtful whether there was any rise of the lever due to the substance added, or whether we were dealing simply with the slow, elastic recoil of the ring which might in any case have taken place. Condensed results of the experiments follow.

Protocols.—Aug. 19, 1912. Citrate plasma gave practically no rise; citrate serum, a good rise.

Aug. 22. Citrate plasma gave practically no rise; the corresponding citrate serum gave a good rise.

Sept. 13. Citrate plasma gave a slight rise; citrate serum, a very good rise; serum without citrate, a very good rise.

Sept. 14. The same plasma and serum that were compared on Sept. 13. Plasma gave no noticeable rise; citrate serum (replacing plasma), a good rise.

Sept. 16. Citrate plasma, no noticeable rise; citrate serum, a fairly good rise, which would correspond to something intermediate between moderate and good in the above scheme. Citrate plasma from blood whipped for fifteen minutes before separating the plasma, as was the case with the defibrinated blood used for comparison, gave no noticeable rise. The object of whipping the blood was to test the question whether in this way any pressor effect could be developed when the blood was whipped after some time had elapsed since it had been shed.

Sept. 19. Citrate plasma, no noticeable rise; serum plus citrate, moderate rise; serum from clot, very good rise; serum from clot diluted with an equal volume of Ringer, good rise.

Sept. 21. Citrate plasma, very slight rise (less than would correspond with a slight rise in the above scheme); citrate serum replacing plasma, slight rise; citrate serum replacing Ringer, moderate rise; serum from clot, good rise.

Sept. 24. Citrate plasma, slight rise; citrate serum, good rise; serum without citrate, very good rise.

Oct. 16. The details of this experiment are given in the text (p. 162).

Perfusion Preparation.—On this preparation, also, the results were more uniform than with the only other plasma used for perfusion,—hirudin plasma. Whenever serum gave a marked slowing in the flow, the slowing produced by the corresponding plasma was decidedly less. Yet the plasma invariably produced some slowing, and where the serum effect was small, the relative difference between the action of the plasma and serum was less than where the serum effect was large. The smaller the effect produced by the serum, the greater the tendency for the difference between it and the plasma effect to disappear. For this reason only experiments in which serum gave a decided effect were looked upon as available for deciding the question at issue.

Figure 2 gives in the form of curves an example of the results for one specimen of the citrate material. Small portions of the actual drop records are reproduced in figure 4.

Intestine.—The intestine preparation, on the other hand, showed no constant difference between the citrate plasma and serum. With each, the first effect was a rapid increase of tone, which, however, was much more transient than that given by plasma or serum not containing citrate. The rise quickly gave place to a diminution of tone which in some cases sank far below the initial level, the strength of the beats being at the same time diminished. There is every reason to believe that the initial increase of tone represents the effect of the plasma and serum as such, and the subsequent depression the effect of the citrate, which more or less completely neutralizes the tone-increasing power of the serum and plasma as indicated by control observations with citrate-Ringer solution. Since the citrate action in the concentration used masks to a great extent the tone-increasing power of the serum or plasma, the comparison on the intestine preparation is not quite so satisfactory as with the other plasmas and sera. Yet there is no evidence of any inferiority of the plasma as compared with serum in its power to increase the tone of this smooth muscle preparation in the presence of the opposite effect of the citrate. Indeed some of the tracings might seem to indicate that the plasma may have an even greater effect than the serum in neutralizing the depressant action of the citrate. We should hesitate, however, to conclude

that the plasma is ever superior to the serum in this regard, as it is doubtful whether the differences noted in certain observations do not fall within the normal limits of variation.

Uterus.—For the uterus preparation also, the plasma and serum show practically the same behavior. This statement is based on an examination of the citrate material of August 19, September 16, and September 24. Specimen tracings are reproduced from the experiment of September 24 for the intestine (figure 6) and for the uterus (figure 7). In the experiment of September 16 the preliminary rise of tone in the intestine was better marked, in that of August 19 less marked, than in the tracings reproduced both with plasma and with serum. But the way in which the tone is sustained against the depressant influence of the citrate (observation 16, figure 6) is even better evidence than the absolute height of the preliminary peak that the tone-increasing power of the plasma and serum is still in evidence, even in the presence of the citrate, and to an equal degree in both plasma and serum.

It was not considered advisable to test on the uterus and intestine the citrate material of the other experiments which had been used for the ring and perfusion observations simply because it was recognized that the presence of the citrate rendered the liquids less suitable for work with these objects. Only a sufficient number of experiments were made to demonstrate that in the case of the citrate material as well as in the case of the hirudin and peptone material, approximate equality of effect was obtained from plasma and serum. This was considered important since the most striking difference between the plasma and serum in the case of the artery rings and the perfusion preparation had been made out with the citrate material.

PEPTONE PLASMA AND SERUM.

Material was obtained from two dogs. Material from two other dogs was not used on account of early clotting.

Experiment of Aug. 22, 1912.—After about 50 c.c. of blood had been drawn from one carotid artery of a dog, and defibrinated, 0.7 gm. per kilogram of Witte's peptone was injected into the femoral vein. A 10 per cent. solution of the peptone made up in a 0.9 per cent. sodium chloride solution was used. Blood was then drawn in twenty-five minutes through a cannula (boiled and oiled) in the other carotid. The plasma from this blood although it clotted later

showed no signs of coagulation before or during the vessel ring experiment. The effect on the ring was fully as great as that of the serum.

Experiment of Aug. 24, 1912.—From one carotid of a dog blood was drawn and defibrinated. Then 0.7 gm. of peptone per kilo of body weight was injected intravenously as in the preceding experiment. The injection (58 c.c.) was made in two minutes (2:24 to 2:26 P. M.). Six samples of blood were then drawn off from the other carotid at the following times: (1), 2.29; (2), 2.32; (3), 2.36, (4), 2.51; (5), 3.10; (6), 3.20 P. M.

Artery Rings.—The effect of most of the specimens of peptone plasma was as great as that of serum from the same animal. In two observations the peptone plasma produced a somewhat greater effect than the serum. In two observations the action of the plasma was somewhat inferior to that of the serum. Obviously the results of the comparison between peptone plasma and serum are very different from the corresponding results for citrate material. This is well illustrated in the following condensed protocol showing the results of the ring tests with the material of August 24.

In this experiment the carotid of a dog was used for the artery rings, the only occasion on which an artery other than the sheep's carotid was employed. The rings being smaller, the rise observed was not so great. Nevertheless the effects were prompt and well marked. The results of comparing the various plasmas with serum from the same dog may be summarized as follows:

Plasma 1.—Not as great as serum.
Plasma 2.—About the same as serum.
Plasma 3.—Had clotted before the tests were made.
Plasma 4.—Fully as great as serum.
Plasma 5.—Not quite equal to serum.
Plasma 6.—Had clotted before the tests were made.
None of the material used for the ring tests had shown any clotting.

No observations were made with the peptone material on the perfusion preparation.

Intestine.—For comparison with the peptone plasma it was necessary to use serum either undiluted or diluted with Ringer to a known extent. Peptone was not added to the serum, as the amount of peptone in any plasma was unknown. Since the plasma was obtained by a reaction within the body,—a veritable intoxication indeed,—exact comparison of plasma and serum was in any case rendered impracticable by the fact that in addition to the substance which restrains clotting other substances formed in the reaction of the organism to peptone may be present in the plasma and may

affect the intestine and uterus. Nevertheless the general resemblance between the action of peptone plasma and serum on these test objects is not obscured, as is shown in the tracings reproduced in figures 8 to 11. It is worthy of note that plasma 5 (figure 10, observation 18), which clotted before it was used, instead of causing a larger increase of tone than plasma 1 or 4, produced a considerably smaller increase. Too much stress, however, must not be laid on this, for plasma 2 (figure 10, observation 12), which had not clotted, produced an even smaller effect, and therefore an effect much inferior to that of the other unclotted plasmas. The cause of this difference in the effects was not due to a deterioration of the segment, since after these observations it still gave almost as great an increase of tone with the serum as at first. As already remarked, the changes in the blood by the reaction of the organism to the peptone could not be controlled and these probably influenced the action of the various specimens of plasma in different degrees. The peptone itself, as shown in figure 11, caused some inhibition of the segment, and this might also be a factor in producing the differences between the specimens, although the presence of unchanged proteose cannot itself account for them, since it is to be supposed that more would be found in plasma 1 than in plasma 2.

HIRUDIN PLASMA AND SERUM.

Experiments were made with material obtained from eight animals.

Artery Rings.—Hirudin plasma always gave some effect except with the least sensitive preparations. Occasionally the effect of the plasma was marked. In no instance, however, did the plasma produce as great an effect as the serum, while in general the plasma effect was distinctly inferior (figure 1, observations 1 to 5). Yet on the whole the difference between the hirudin plasma and serum was less than the difference between the citrate plasma and serum. No clotting was ever observed in the plasma during contact with the rings, although carefully looked for. While some of the preparations clotted later, this did not occur for many hours. Most of them did not clot during the period for which they were observed (several days in the ice chest).

Protocols.—Aug. 29, 1912. Dog blood was run into 25 c.c. of hirudin solution (1 mg. per c.c.) to make 100 c.c. For the serum 60 c.c. of defibrinated blood was mixed with 20 c.c. of the hirudin solution.

Results of the Ring Tests.—Plasma, moderate rise; hirudin serum, good rise; serum from defibrinated blood, good rise.

Sept. 10, 1912. (1) Plasma. Blood was run into 20 c.c. of hirudin solution (1 mg. per c.c.) to make 100 c.c.

(2) Serum. 80 c.c. of defibrinated blood were mixed with 20 c.c. of hirudin solution.

(3) Same as (1); collected after (2).

(4) Same as (2); collected after (3).

(5) Serum from defibrinated blood; collected after (4).

(6) Serum from clot; collected after (5).

Great care was taken in collecting the hirudin blood and no flaw was discoverable in the technique.

Results of the Ring Tests.—Plasma, good rise; hirudin serum, very good rise, more than with plasma; hirudin solution, no effect; serum without hirudin, very good rise, about the same as with hirudin serum.

Sept. 13, 1912. Plasma. 40 c.c. of blood from a dog's carotid were run into 20 c.c. of hirudin solution (1 mg. per c.c.).

Hirudin serum. 20 c.c. of hirudin solution were added to 40 c.c. of defibrinated blood.

Results of the Ring Tests.—Plasma, slight rise; hirudin serum replacing plasma, very good rise; hirudin replacing Ringer, very good rise. Same material tested on Sept. 14. Hirudin plasma, slight rise; hirudin serum, good rise.

Sept. 26, 1912. Blood was obtained from two rabbits, with the usual precautions. From each rabbit 30 c.c. of blood were run into 10 c.c. of hirudin solution (1 mg. per c.c.). To defibrinated blood from the same animals (a mixture of approximately equal quantities of blood from each rabbit) a corresponding amount of hirudin solution was added. The two specimens of unclotted hirudin blood first obtained were mixed. The preparations were kept in the incubator for about thirty minutes before centrifugalization.

Results of the Ring Tests.—Plasma, moderate effect; hirudin serum displacing plasma, good effect; hirudin serum displacing Ringer, very good effect; hirudin solution in Ringer (dilution the same as in the plasma, as indicated by the relative volume of corpuscles and plasma in the blood), no effect.

Oct. 2, 1912. Plasma. 60 c.c. of dog blood were run into 20 c.c. of hirudin solution (1 mg. per c.c.).

Hirudin serum. 60 c.c. of defibrinated blood were mixed with 20 c.c. of the hirudin solution.

Results of the Ring Tests.—Plasma, moderate rise; hirudin serum, good rise; plasma (ring stretched in hirudin Ringer solution instead of in Ringer), moderate rise, but no smaller than that given by the plasma replacing Ringer.

On Oct. 3, 1912, the unclotted hirudin blood of Oct. 2 was diluted with such a volume of Ringer as made with the hirudin solution previously added a final dilution of the blood with its own volume of solution. The same was done

with the defibrinated blood. The dilute plasma and serum were then separated and tested on artery rings with the following results; serum, good rise; plasma, slight rise; serum displacing plasma, good rise; plasma, slight rise; serum displacing plasma, good rise.

In this experiment the relative activity of serum and plasma in the dilution mentioned resembled more closely the relative effects of citrate plasma and serum, which were always obtained from blood diluted to the same extent.

Since the hirudin plasma and serum obtained in our experiments were less diluted than the citrate plasma and serum it seemed desirable to ascertain whether this factor had any influence in producing the difference noted in the relative effect of the plasma and serum in the two cases. Figures 12 and 13 illustrate observations made for this purpose with the material of October 16. In figure 12 at 1, dog's citrate plasma, of the concentration ordinarily used in our experiments (that is, obtained from blood diluted with its own volume of citrate solution) and referred to in the description of the figure as plasma A, replaced Ringer and was itself replaced at 2 by the corresponding citrate serum (serum A). At 3 citrate plasma (plasma B), from the same animal obtained from blood diluted with half its own volume of a Ringer citrate solution containing twice the concentration of citrate ordinarily used for plasma, replaced Ringer, and was itself replaced at 4 by the corresponding citrate serum (serum B). It will be seen that in the greater concentration the plasma and serum effects are increased in approximately the same proportion. Accordingly, the greater concentration of the hirudin material as ordinarily tested does not of itself account for the difference referred to. Observations 5 and 6, and 7 and 8, were repetitions respectively of observations 1 and 2, and 3 and 4, on rings from the same artery. Although the absolute amount of the rise is different, yet the proportion between the serum and plasma effect is not greatly altered and is still of the same order of magnitude as we have always observed with the citrate material.

On rings of another sheep artery (figure 13) observations 14 and 15, corresponding to 1 and 2, and observations 16 and 17, corresponding to 3 and 4, of figure 12 show even more clearly that so long as the concentration is kept the same in both a mere difference of concentration in the plasma and serum does not conceal the

great difference in their pressor action. For comparison observations 18 and 19 (figure 13) are reproduced, showing the effect of the citrate serum in the two dilutions when the Ringer was directly replaced by it. In figure 14 with rings from a third sheep's artery, the effects of serum from the same dog (without citrate), diluted with different amounts of Ringer, are displayed. The details are given in the description of the figure.

Perfusion Preparation.—Hirudin plasma and serum differ less in their effect on the rate of perfusion than do citrate plasma and serum. In certain cases the effect of the plasma, although the maximum slowing was as great as that produced by the serum, was less durable. In a number of instances the total plasma effect was fully as great as the serum effect. No indication of clotting was ever seen in the diluted plasma which was injected. Figures 2 and 3 give specimens of the curves showing the changes in outflow in two of the experiments (those of September 26 and October 2). Figure 5 shows a specimen of the drop record from the experiment of September 26.

Intestine.—No appreciable difference was found in the effect of hirudin plasma and serum on this preparation. This confirms the conclusion previously reached by one of us.[10] Only two experiments, one on dog blood and the other on human blood, were referred to in that paper, but these were part of a larger series which was continued in the present investigation. It may be well to explain that in these two experiments Ringer's solution without hirudin was added to the defibrinated blood, to give the same dilution as in the case of plasma. This was done with the idea of permitting a more exact appraisal of the advantages of plasma as compared with serum for the detection of adrenalin in the peripheral blood with the test objects (intestine and uterus) previously employed, since in the previous work serum alone had been used. For the purpose of comparing the effects of plasma and serum on the tone of the intestine and uterus segments, the absence of hirudin in the defibrinated blood in these two experiments really rendered the tests more severe, since the hirudin solution itself (in Ringer) caused a slight fall of tone of the intestinal segment, with some diminution

[10] Stewart, G. N., *Jour. Exper. Med.*, 1912, xvi, 502.

in the size of the beats. In all the other experiments hirudin was added to the defibrinated blood in the same proportion as to the whole blood. The results were identical. In no instance was there any distinct and constant preponderance of effect on the part of the serum.

That this was not due to the effect of the serum being a maximal response on the part of the preparation was shown (1) by obtaining markedly greater effects on the same preparation with undiluted serum, and (2) by comparing the effects of serum diluted sufficiently to cause a response much below the maximal with that of plasma diluted to the same degree. The effects were practically identical with the diluted plasma and serum (figures 16 and 17).

A fact of prime importance to be borne in mind in interpreting the tracings from the intestine preparation, and one that was noted in previous papers, is that not infrequently the first observation, or occasionally the first two or three observations, show a much smaller response, at any rate as regards increase of tone, than subsequent observations. This is especially the case when the segment is beating feebly in Ringer solution. It then seems to require some time when the Ringer is replaced by serum or plasma to attain the nutritive condition in which it responds readily to the tone-increasing property of the serum or plasma, the first effect of the serous liquid being to increase the size of the beats. After this stage it will go on responding for a long time through many successive observations with remarkable steadiness. If now with such a preparation a comparison of plasma and serum were begun by the addition of plasma (as in observation 2, figure 17) the change of tone would appear far inferior to that occasioned by a subsequent application of serum, and if the experiment stopped here (before observation 6, for example, had been made in figure 17), the same relation between hirudin plasma and serum as regards their effect on the intestine might be supposed to be demonstrated which undoubtedly exists for citrate plasma and serum in the case of artery rings. Yet this impression would be at once corrected by performing the observations in the reverse order (as in observation 33, figure 18, or observation 2, figure 19). The serum would now appear to increase the tone of the segment far less than the plasma, an equally erroneous

conclusion, as shown by observation 37, figure 18, with the same serum.

Uterus.—For the uterus the same general statement may be made as for the intestine in regard to the action of the hirudin material. The parallelism is even more impressive in the case of the uterus because it exists not only where the uterus responds to serum by an immediate increase of tone, but also where the primary effect of the serum is an inhibition. Thus in figure 20 the effect of the hirudin plasma from rabbits of September 26 on rabbit uterus (observation 39) is to cause practically the same immediate increase of tone as the corresponding hirudin serum (observation 41). That this is not because both effects are maximal is shown by observations 47 and 49 in which the hirudin plasma and serum were each diluted with five volumes of Ringer. The increase of tone produced by the dilute plasma is here, indeed, greater and somewhat more durable than that produced by the dilute serum. In figure 21 dog hirudin plasma (observation 28) and hirudin serum (observation 26) of September 12 caused practically identical and very prompt increases of tone in a rabbit uterus segment, the increase in each case being somewhat greater than that occasioned by the serum of the same dog without hirudin but diluted with its own volume of Ringer.

In figures 22 and 23, on the other hand, material obtained from a dog on August 29 caused in a rabbit uterus preparation as its first effect a marked relaxation. Observations 24 and 28 (figure 22) are with hirudin plasma, and observation 26 is an intermediate observation with the hirudin serum. The general similarity between the plasma and serum effects is well marked. Observation 30 (figure 23) shows the effect of the hirudin solution in Ringer, observation 32 that of serum from the defibrinated blood without hirudin, observation 36, the effect of adrenalin 1 : 1,000,000 in Ringer, and observation 42 that of adrenalin 1 : 100,000 in Ringer. The adrenalin observations lend no support to the idea that the inhibition might be an effect of epinephrin in the blood. The inhibitory effect of the serum on this uterus will obviously be intensified by that of the hirudin in the hirudin plasma and serum. The uterus was from an adult non-pregnant rabbit, as was also the uterus used for figures 20 and 21.

TRANSUDATES AND EXUDATES.

The results of these observations are in harmony with those on serum and plasma. Hydrocele and ascitic fluids, presenting the classical characters of non-coagulable transudates, produced no effect on artery rings. Only the hydrocele fluid was tested on the perfusion preparation. It caused merely a slight slowing in the flow, far less than that caused by the serum used for comparison. On the other hand, its action on the intestine closely resembled that of the blood serum and was at least as great as that of the serum from a typical inflammatory exudate (pleural liquid) which had clotted into a jelly before it was used. For further details and for the action of transudates on the uterus preparation the following paper may be consulted.[11]

SERUM FRACTIONS AND MODIFICATIONS OF SERUM.

All that need be said at present about these observations is that it proved possible to obtain (1) alcoholic extracts from serum which had no effect upon artery rings and yet caused in the intestine and uterus segments qualitatively the same changes as the original serum (figures 24 and 25), and sometimes quantitatively even greater effects, and (2) modifications of serum (heat preparations) which while differing markedly in their quantitative effect on the intestine and uterus preparations exerted on the artery rings approximately the same effect (figures 24 and 25). This supports the conclusion already drawn from the comparison of serum and plasma that one and the same material may affect the various test objects very differently, and shows that the existence of a marked difference in the action of a given plasma and serum on the artery rings, for example, does not render it *a priori* probable that a similar difference will be found in their action on intestine segments. Further illustrations of this are found in some observations on the action of certain foreign proteins on some of the test objects.

Protocols.—Alcohol extracts. July 19, 1912. 100 c.c. of sheep serum were mixed with 400 c.c. of 95 per cent. alcohol. The mixture was stirred occasionally for five minutes and then filtered with suction. The filtrate was evaporated on

[11] Stewart, G. N., and Zucker, T. F., *loc. cit.*

a boiling water bath.[12] The residue was taken up with 100 c.c. of Ringer's solution and labelled A(1).

After precipitating another portion of the serum, filtering, and evaporating as in the preparation of A(1), the residue was taken up with successive portions of absolute alcohol. After filtering, this alcoholic extract was evaporated and the residue taken up with a volume of Ringer solution equal to the original volume of the serum (A(2)).

July 25, 1912. An extract of another sample of sheep serum was made in the same way as A(2) of July 19. It was marked A(3).

Results of the Ring Tests.—July 23. A(1), no rise; serum, good rise, A(2), no rise; serum, good rise.

July 25. A(1), no rise; serum, moderate rise; serum diluted with four volumes of Ringer, moderate rise.

July 26. A(3), no rise; serum, very good rise.

Intestine and Uterus Tests.—A(1), A(2), and A(3) all produced a good increase of tone, whether tested at once or after being kept for several days. The effect on the intestine, however, was usually less, and was always less durable than that caused by the original serum (figure 24, observation 4), while on the uterus the effect might be decidedly greater than that of the serum (for example the effect of A(3) in observation 16, figure 25). In all the observations in which it was used A(3) gave with the uterus an effect greater than that of the serum, and this is seen in experiments on the uteri of two rabbits separated by an interval of two days (July 25 and 27).

Heat Preparations.—July 19, 1912. 100 c.c. of sheep serum were heated in a boiling water bath. After coagulation, 100 c.c. of Ringer solution were added, and the mixture was rubbed up and strained through cloth. The filtrate was labelled H(3).

July 25, 1912. To 50 c.c. of sheep serum obtained on July 25, 200 c.c. of Ringer solution were added and the mixture was heated on wire gauze to boiling. Boiling was kept up for a few minutes. The liquid was then strained through cloth but hardly any coagulum had formed and it passed through as an opalescent liquid. The filtrate was labelled H(5).

Fifty c.c. of the same serum were coagulated in a boiling water bath. The coagulum was rubbed up in a mortar with 200 c.c. of Ringer solution. The solid particles were strained out and the liquid was filtered. The filtrate was labelled H(6).[13]

Results of the Ring Tests.—July 24, 1912. H(3) gave a good rise, about the same as that given by the serum used for comparison.

July 26. H(5), good rise; serum diluted with four volumes of Ringer, not as good a rise as with H(5), but the difference is slight; H(6), about the same as serum diluted with four volumes of Ringer.

[12] Alcohol extracts evaporated at lower temperatures and reduced pressure have a good pressor effect on artery rings.

[13] This protocol is not quoted as a method by which one may obtain with certainty from any serum preparations differing in their action as H(5) and H(6) did. Our point is that these products, however obtained, did in fact show a very different action on the various test objects.

Intestine.—H(3) gave a very good and sustained increase of tone quite comparable to that occasioned by the corresponding serum. H(5) (figure 24, observation 15) caused a large increase of tone greater than that produced by the unheated serum similarly diluted with Ringer (observation 9). The contractions disappeared and the increase of tone was soon succeeded by a great fall. Not only quantitatively but qualitatively the effect was quite different from that of the unaltered serum and from that of H(6). This was verified by repeated observations on intestine segments from two rabbits.

H(6) (figure 24, observation 11) produced a rise in the curve somewhat inferior to that produced by the unheated serum similarly diluted. The beats continued just as in the unaltered serum and the increase of tone was well sustained. This similarity in the action of H(6) with that of serum was also verified by a number of observations on preparations from two rabbits. The difference between H(6) and H(5) is most strikingly shown in observations 17 to 20, figure 24, where the replacement by H(6) of serum diluted with four volumes of Ringer produced little effect, while the subsequent replacement of H(6) by H(5) caused the characteristic diminution of tone after a preliminary rise.

Uterus.—H(3) affected the uterus preparation much as the corresponding serum did, causing the usual increase of tone. H(5) produced a decidedly greater and more sustained increase of tone than either H(6) or the unaltered serum correspondingly diluted.

FOREIGN PROTEINS.

As already indicated, the main point of interest in these observations as regards our present subject is the further and, indeed, striking evidence they afford that a material which exerts little or no action on one of the test objects (artery rings) in comparison with serum may have a very decided effect, quite comparable in magnitude with that of the same serum on another test object (intestine segment).

Protocols.—Sept. 28, 1912. Egg white. The white of an egg which was laid the same day and in which decomposition products are therefore certainly excluded was strained through several layers of cheese cloth and various dilutions of it with Ringer solution were made.

Witte's Peptone.—One gm. of Witte's peptone was rubbed up with 20 c.c. of Ringer solution. A slight sediment remained behind on decanting. Further dilutions were made with Ringer solution.

Wheat Flour Suspension.—Schultz states that an extract of the wheat proteins gives a better effect on the guinea pig intestine, but as he states that he also obtains a good effect with a suspension of wheat flour in distilled water we used the latter. This suspension was made by shaking up 25 gm. of wheat flour with 50 c.c. of distilled water. Further dilutions were made with Ringer solution.

Results of the Ring Tests.—Egg white, undiluted, slight effect; diluted with one volume of Ringer, very slight effect (figure 1, observation 14); rabbit serum diluted with one volume of Ringer, very good effect (figure 1, observation 16). Dog serum (of Sept. 24) also gave a very good effect. These observa-

tions should be compared with the corresponding observations on the intestine preparation (figure 26). It will be seen that the tone-increasing power of the egg white diluted with its own volume of Ringer on the intestine preparation is of the same order of magnitude as that of the rabbit serum correspondingly diluted.

Witte's Peptone.—Both the 5 per cent. solution and a 0.5 per cent. solution were practically without effect on the artery rings.

Wheat Flour Suspension.—50 per cent. suspension; slight effect. Suspension diluted with 9 volumes of Ringer; very slight effect. On the intestine preparation, the 50 per cent. suspension produced a fair increase of tone, which, however, was transient.

Intestine.—Solutions of egg white which had no effect on the artery rings caused a decided increase of tone on the intestine preparation, an increase of tone comparable with that produced by serum (observations 27 and 29, 31 and 33, figure 26).

Solutions of Witte's peptone caused some inhibition of the intestine, which gave place to a remarkably abrupt increase of tone on washing with Ringer's solution (observations 31 and 35, figure 11). No such phenomenon was observed with the artery rings. It does not seem to depend on a difference between the effects of concentrated and dilute solutions of the peptone, as we at first thought might be the case from the statement of Schultz[14] as to the stimulating action of peptone on guinea pig intestine. A possible explanation, but one which we have not tested, is that Witte's peptone contains two substances which act on the intestine, one inhibitory and the other stimulating. The first substance (or its action) is easily removed by washing with Ringer, while the second substance (or its action) is not so easily removed by washing.

Extracts of wheat flour which caused some increase of tone in the intestine preparation were inactive as regards artery rings.

A word or two may be added on the bearing of our results on the technique of epinephrin tests. We believe that Janeway and Park[15] are correct in considering the artery ring method a good one for this purpose, although we are not impressed with the sensitiveness of the ring preparations for adrenalin. Our experience, like that of most previous observers, is that artery rings are inferior in this respect to preparations such as intestine and uterus segments. The great advantage of the artery ring in our opinion is the rela-

[14] Schultz, W. H., *Bull. Hyg. Lab., U. S. P. H. and M.-H. S.*, 1912, No. 80, 37.
[15] Janeway, T. C., and Park, E. A., *Jour. Exper. Med.*, 1912, xvi, 541.

tive constancy of the response in a qualitative sense and its definiteness. The response to epinephrin as well as to serum is, from the nature of the biological mechanism involved, an exceedingly simple, and therefore unambiguous one. The intestine or uterus segment has several ways in which its activity may be altered. The beats may increase or diminish in strength or in rate, and the tone may increase or diminish. The response to adrenalin or serum in one condition of a segment may be different from the response in another nutritive condition. Another advantage of the rings is that the complicating effect of the pressor action of serum can, as a rule, be avoided to a considerable extent with the artery preparation by using citrate plasma. This as we have shown is not the case for intestine and uterus segments.

The use of plasma, however, even for the rings has its disadvantages, of which the gravest is that we cannot be quite certain, even with citrate plasma, that a given pressor effect is due to epinephrin and not to a development, greater than the ordinary for this form of plasma, of the pressor property of shed blood. This difficulty becomes greater with the Laewen preparation even when citrate plasma is used, and still greater, both with the rings and the perfusion preparation, when hirudin plasma is employed. We are therefore impressed by the argument of Kahn in which he deprecates the abandonment of serum for the perfusion method in favor of plasma, as recommended by O'Connor, and points out that, although the constrictor effect of a serum may often be greater than that of the corresponding plasma, it is much more constant and can accordingly be more easily taken account of. For the intestine and uterus preparations, as we have shown, plasma offers no advantages over serum. Anyone who desires to work with these preparations on account of their superiority in sensitiveness to the other preparations will therefore naturally employ serum. Hence we fail to see any reason for the statement of Janeway and Park that "Stewart's negative conclusions are invalidated by the fact that he worked with serum." The further statement of these authors, that "Stewart obtained epinephrin reactions constantly only when the adrenal gland was handled roughly or massaged, and regularly failed to obtain such reactions when precautions against

this were taken even though the splanchnics were stimulated," is quite erroneous and doubtless based on some misapprehension.

DISCUSSION AND SUMMARY.

Apparently, then, we are confronted with this result,—that citrate plasma which causes little or no constriction of the stretched artery ring, little or no slowing of the flow through the frog perfusion preparation, while the corresponding serum produces a marked effect on both preparations, will affect the intestine or uterus preparation practically in the same way as the corresponding serum. Hirudin plasma and serum exert on the intestine and uterus preparations practically the same effect, causing a marked increase of tone. On the artery ring preparation, there is a difference although it is not so strongly marked as in the case of the citrate material. The frog perfusion preparation, as regards the effect of the hirudin material, seems to occupy an intermediate position between the intestine and uterus on the one hand, and the artery ring on the other.

Of the three plasmas, the peptone plasma most closely resembles serum in its action on the artery rings. Like the other plasmas its effect on the intestine and uterus does not differ appreciably from that of the serum. From these observations the following conclusions seem justified.

A change occurs in shed blood which confers on it the property of constricting artery rings and of slowing the flow through the perfused frog preparation. If this property is in any degree possessed by circulating blood it is at least markedly increased after the blood is shed. The change, whatever it may be, does not entail any essential alteration in the action of the blood on intestine and uterus segments. The tone-increasing property developed in the shed blood may, therefore, so far as the four test objects included in the present survey are concerned, be looked upon as especially affecting the blood vessels and probably their smooth muscle directly. This need not imply that the pressor substance, if it is a single definite substance developed in the shed blood, exerts no action on the smooth muscle of the intestine and uterus preparations, but merely that its action on these objects is masked by the general action of the serum and plasma, so that in the presence of the other

constituents common to serum and plasma, its effect is inconspicuous or not to be detected at all, while on the blood vessel preparations, especially the artery rings, the effect of the pressor substance is the dominant one, and the general action of the serum and plasma is feeble or undetectable.

It is not the clotting process as such, *i. e.*, the actual change of fibrinogen into fibrin, that is responsible for the differences between serum and plasma revealed by the biological tests employed, but some process which precedes or accompanies the clotting and which may or may not be causally related to it. Changes in formed elements of the blood under the influence of the changed conditions (contact with foreign bodies, restriction of gaseous exchange, etc.), which blood encounters as soon as it leaves the living vessels, are known to occur. Among these it is to be assumed are the changes which condition the differences between plasma and serum under discussion.[16] Even if these changes represent preliminary stages in coagulation (liberation of the factors necessary to the formation of thrombin, for instance), they may still occur to a greater or less extent in blood which is prevented in certain ways from clotting, since it is known that the procedures by which the various noncoagulable plasmas are obtained break at different points the chain of events which normally ends in coagulation. A procedure which simply supplies sufficient antithrombin to neutralize the thrombin which has been allowed to form in normal amount may not interfere at all with the changes in the formed elements, and the pressor property of the resulting plasma may then be as marked as that of the serum. On the other hand, a procedure which hinders clotting by preventing or diminishing the alterations in the cells, for example, the addition of a substance which acts as a preservative for blood platelets, will very likely yield a plasma with little or no pressor effect in comparison with the serum. The mere prevention of clotting, then, except in so far as it is an index of the prevention of changes in the formed elements may have little significance in preventing the development of the pressor property. Indeed it is

[16] Since this was written we have found that extracts of platelets obtained from citrate blood, the plasma of which is totally inactive, exert a strong constricting effect on artery rings.

EXPLANATION OF PLATES.

All tracings are to be read from left to right. Time is marked in half minutes. Unless otherwise mentioned the liquid to be tested was always caused to displace the Ringer solution in which the preparation was immersed. The original relation both to base line and to time trace is preserved in figures 6 to 10, 14, 15, and 19. The relative distance from the base line is preserved in figures 16 to 18, 20 to 23, and 26, but all the curves are brought up closer to the time trace.

PLATE 11.

FIG. 1. Sheep artery rings. For observations 1 to 5 the rabbit hirudin material of Sept. 26 was used; for observations 6 and 7 the dog citrate material of Sept. 24. In observations 14 and 16 a dilution of egg white was compared with a dilution of serum. The arteries were from different animals in the three sets of observations. Of the two time traces in the lower right hand portion of the figure the upper belongs to observation 3, and the lower to observations 6 and 7. The time trace in the lower left hand portion of the figure belongs to observations 4 and 5. The time trace at the top of the figure corresponds to all the other observations. At 1 hirudin plasma replaced Ringer and was itself replaced at 2 by the corresponding hirudin serum. At 3 Ringer was replaced directly by the hirudin serum. At 4 Ringer was replaced by the hirudin solution in Ringer and this at 5 by the hirudin plasma. At 6 Ringer was replaced by citrate plasma and this at 7 by the corresponding citrate serum. At 14 egg white diluted with one volume of Ringer replaced Ringer. At 16 Ringer was replaced by rabbit serum of Sept. 28.

FIG. 2. Curves showing the flow through the frog perfusion preparation. The number of drops per half minute is laid off along the vertical axis and the time in half minutes along the horizontal axis, each small division representing a half minute. Thirty-eight drops correspond to 1 c.c. The upper set of curves was obtained with dog citrate material of Sept. 24, the lower set with the rabbit hirudin material of Sept. 26. The small rise which sometimes occurs immediately after the injection is due to increase of pressure by the injection.

FIG. 3. Curves showing flow through frog perfusion preparation. Hirudin material of Oct. 2. For the upper left hand curve the pressure was 16 cm. of the perfusion fluid; for the others 21 cm.

14
2
1
4
5

FIG. 1.

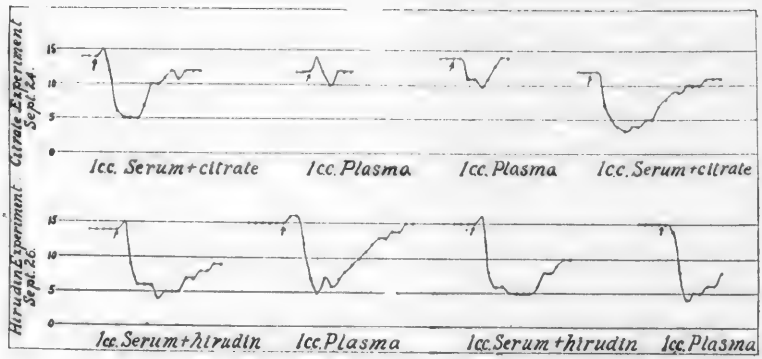

Citrate Experiment Sept. 24.
15
10
5
0
1cc. Serum+citrate
1cc. Plasma
1cc. Plasma
1cc. Serum+citrate
Hirudin Experiment Sept. 26.
15
10
5
0
1cc. Serum+hirudin
1cc. Plasma
1cc. Serum+hirudin
1cc. Plasma

FIG. 2.

I.

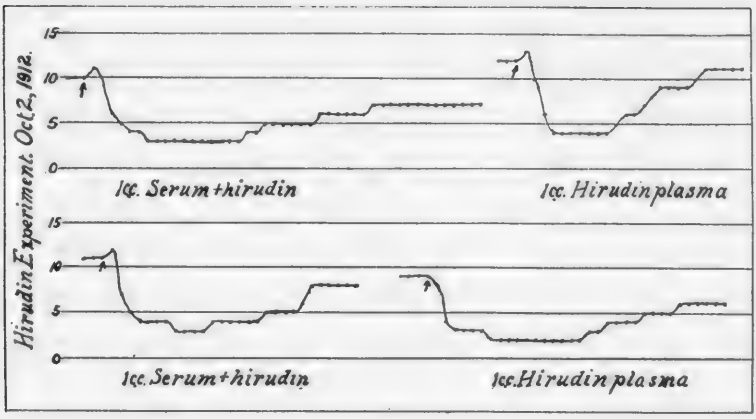


FIG. 3.

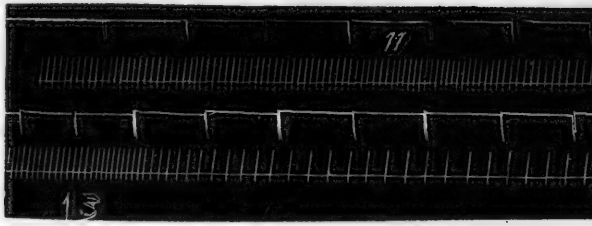
(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 12.

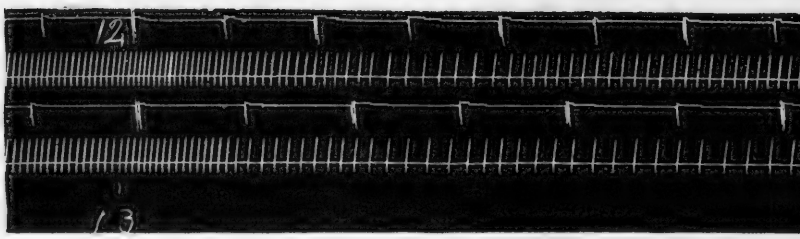
FIG. 4. Tracings showing the change in the rate of drops from the frog perfusion preparation. At 11 citrate plasma of Sept. 24, at 13 the corresponding citrate serum was injected, in each case 1 c.c.

FIG. 5. Tracings showing the change in rate of drops from the frog perfusion preparation. At 12 rabbit hirudin plasma of Sept. 26, at 13 the corresponding hirudin serum was injected, in each case 1 c.c.

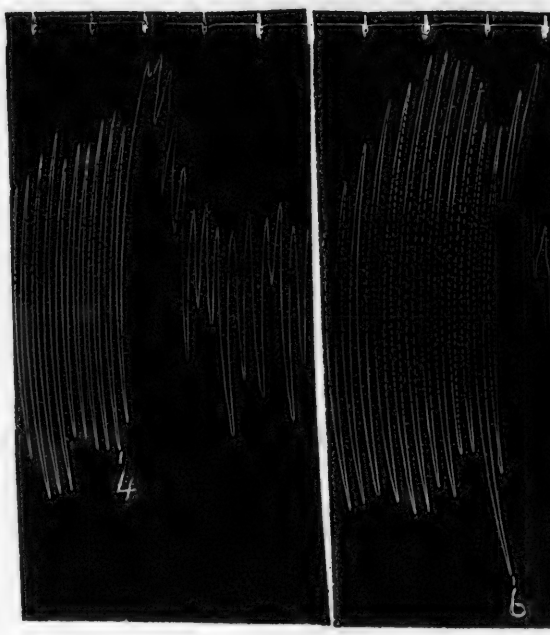
Fig. 6. Intestine. At 4 citrate plasma of Sept. 24, at 6 the citrate serum, at 16 the citrate Ringer solution diluted with its own volume of Ringer replaced the Ringer solution. The magnification of the contraction was about one half of that usually employed.



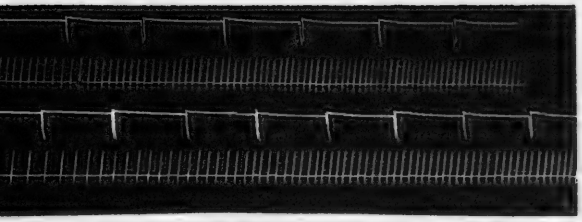
FIG



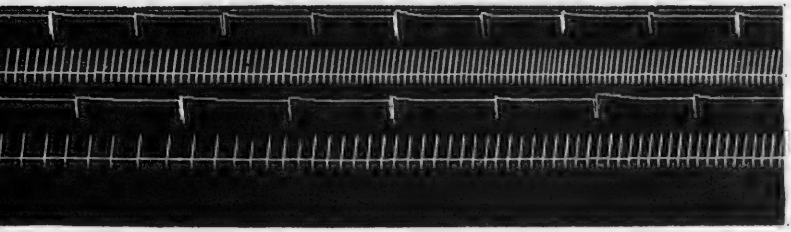
FIG



FIG



4.



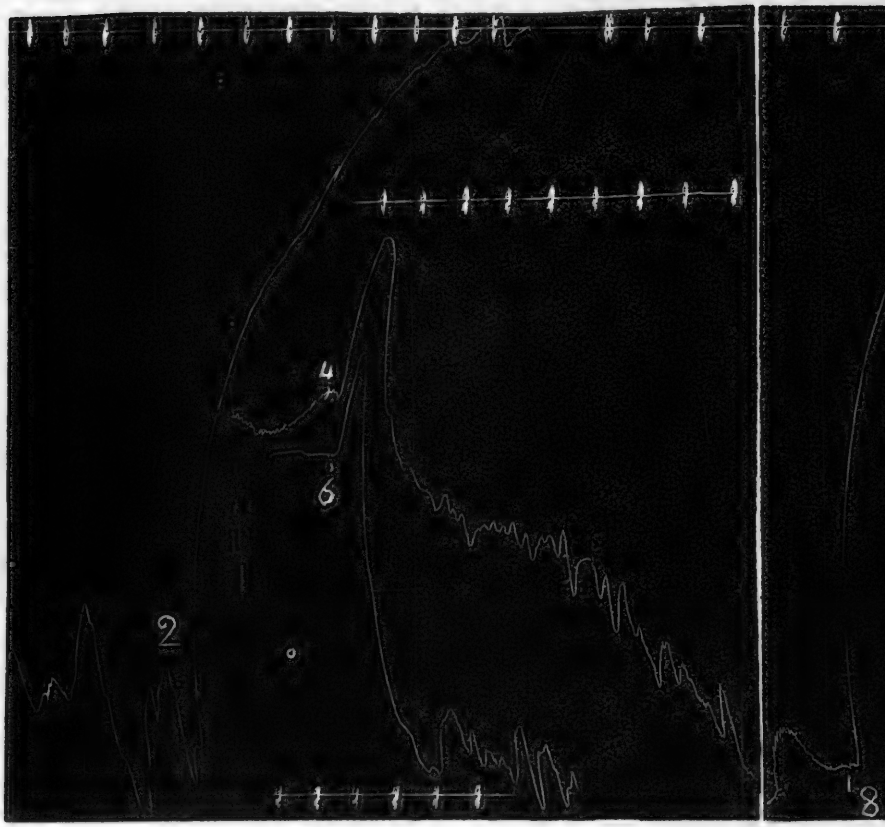
5.

6.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 13.

FIG. 7. Uterus. (From an adult non-pregnant rabbit.) Citrate material of Sept. 24. At 2 serum from clot, at 4 citrate plasma, at 6 citrate serum, at 8 citrate plasma replaced Ringer. All the material was from the same dog. At 11 Ringer was replaced by citrate plasma, at 12 additional citrate plasma was added to replace that removed by the oxygen stream. At 13 the citrate plasma was replaced by citrate serum.


4
6
2
8

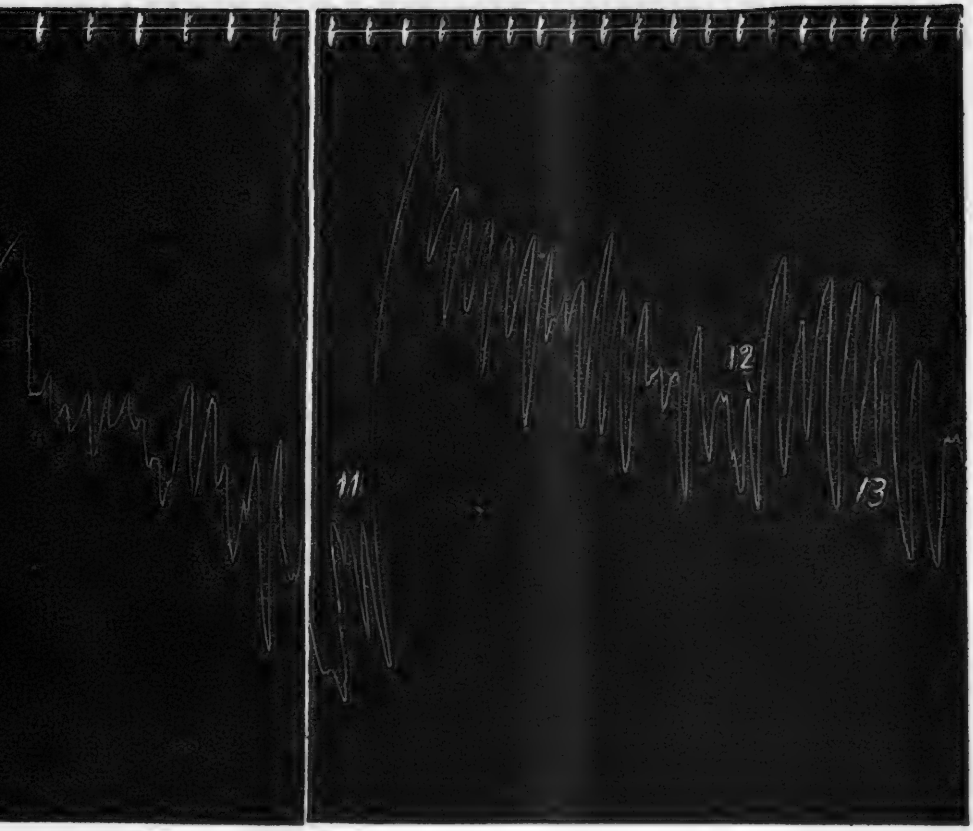


FIG. 7.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 14.

FIG. 8. Intestine. At 2 serum, at 4 peptone plasma 1 of Aug. 24 replaced Ringer.

FIG. 9. The same preparation as used for figure 8. At 6 serum, at 8 peptone plasma 1 replaced Ringer when the initial tone of the segment was considerably higher than in figure 8.

FIG. 10. The same intestine preparation as in figures 8 and 9. At 10 peptone plasma 4 and at 14 serum replaced Ringer. At 12 peptone plasma 2, at 18 peptone plasma 5 replaced Ringer. Plasmas 1, 2, and 4 showed no clotting whatever. Plasma 5 was visibly clotted long before the observation was made.

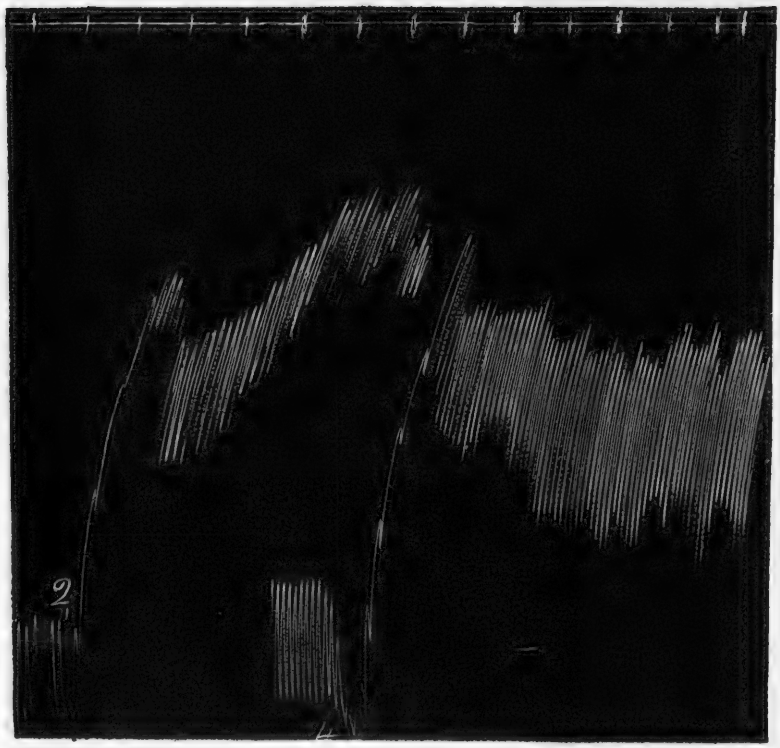
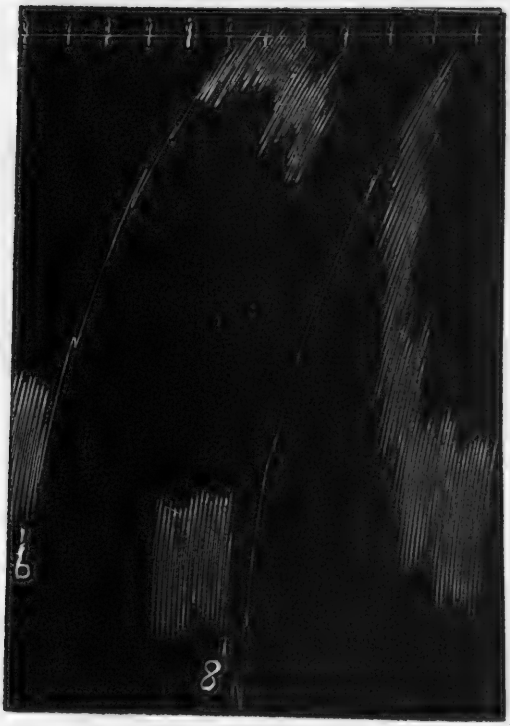


FIG. 8.



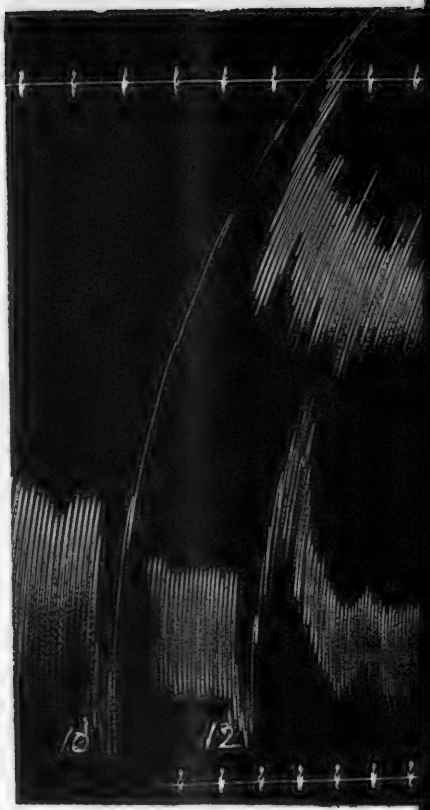


FIG. 9.

FIG. 9.

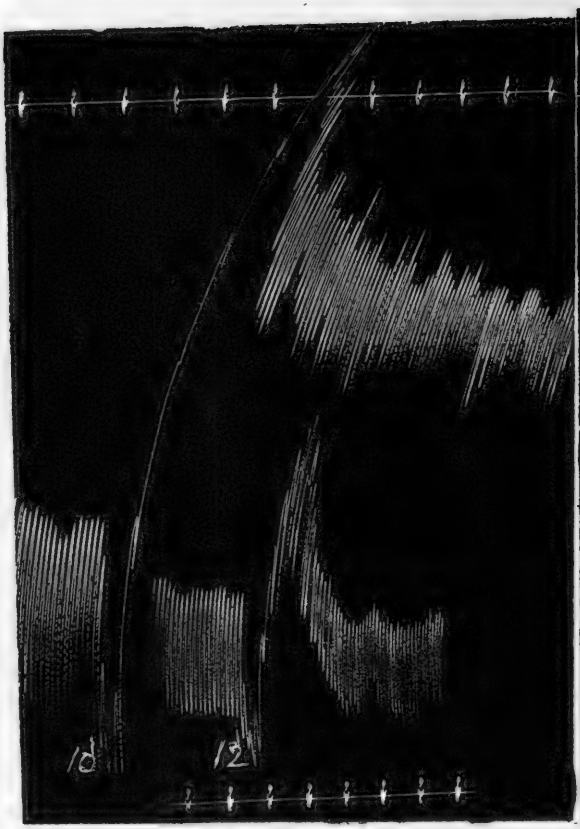
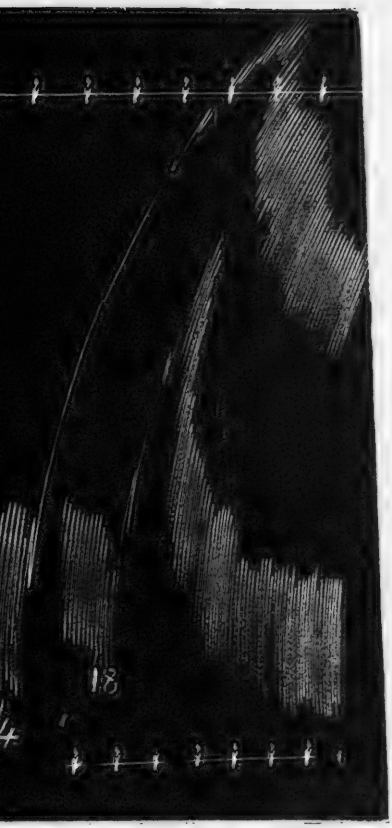


FIG. 10.
(Stewart and

PLATE 14.



ucker: Comparison of Action of Plasma and Serum.)

PLATE 15.

FIG. 11. The same intestine preparation as in figures 8 to 10. At 22 a 1 per cent. solution of peptone in Ringer replaced Ringer. At 30 a 2 per cent. peptone solution in Ringer replaced Ringer. At 31 washing with Ringer was commenced, at 32 suspended, at 33 resumed. At 34 a 10 per cent. peptone solution in Ringer replaced Ringer. At 35 washing with Ringer was begun, at 35a suspended, at 36 resumed, and again commenced at 37.

FIG. 12. Sheep artery rings. Citrate material of Oct. 16 (page 162.) At 1 plasma A replaced Ringer and was itself replaced at 2 by serum A. At 3 plasma B replaced Ringer and was itself replaced at 4 by serum B. 5 and 6 are repetitions of 1 and 2 on another ring from the same artery. 7 and 8 are repetitions of 3 and 4 on another ring from the same artery. The immediate small rise at 8 was due to accidental interference with the thread.

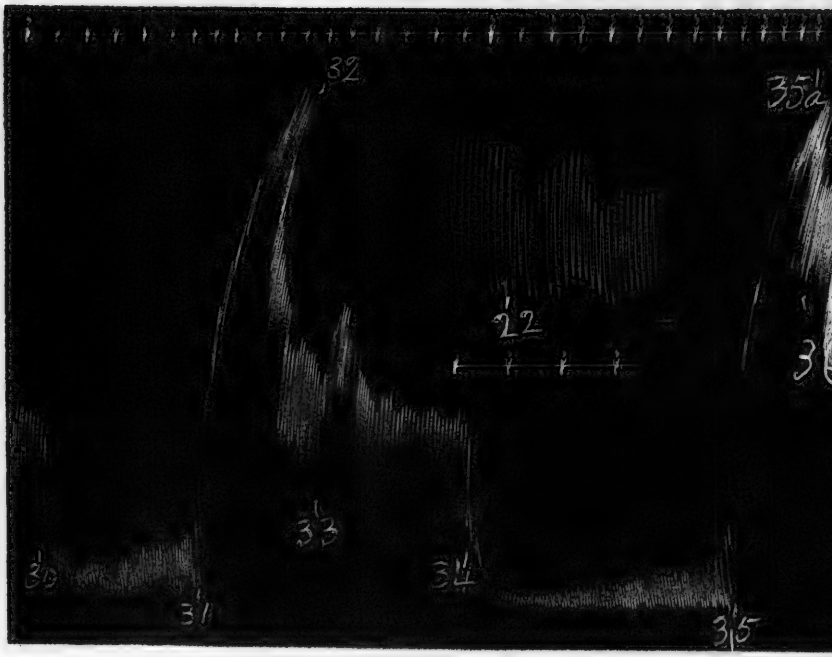


FIG. 11.

37

2
5
3
1

FIG. 12.

E 15.

Serum.)

PLATE 16.

FIG. 13. Sheep artery rings. Citrate material of Oct. 16. Observations 14 to 17 correspond to observations 1 to 4 in figure 12, but were done with rings from another sheep's artery. At 18 serum A replaced Ringer. At 19 serum B replaced Ringer. The upper time trace belongs to observations 16 and 17, the lower to observations 14, 15, 18, and 19.

FIG. 14. Sheep artery rings. Dog serum of Oct. 16 without citrate. At 9 Ringer was replaced by undiluted serum, at 10 by serum diluted with 1 volume of Ringer, at 11 by serum diluted with 2 volumes of Ringer, at 12 by serum diluted with 8 volumes of Ringer. A repetition of observation 12 on another ring gave precisely the same result.

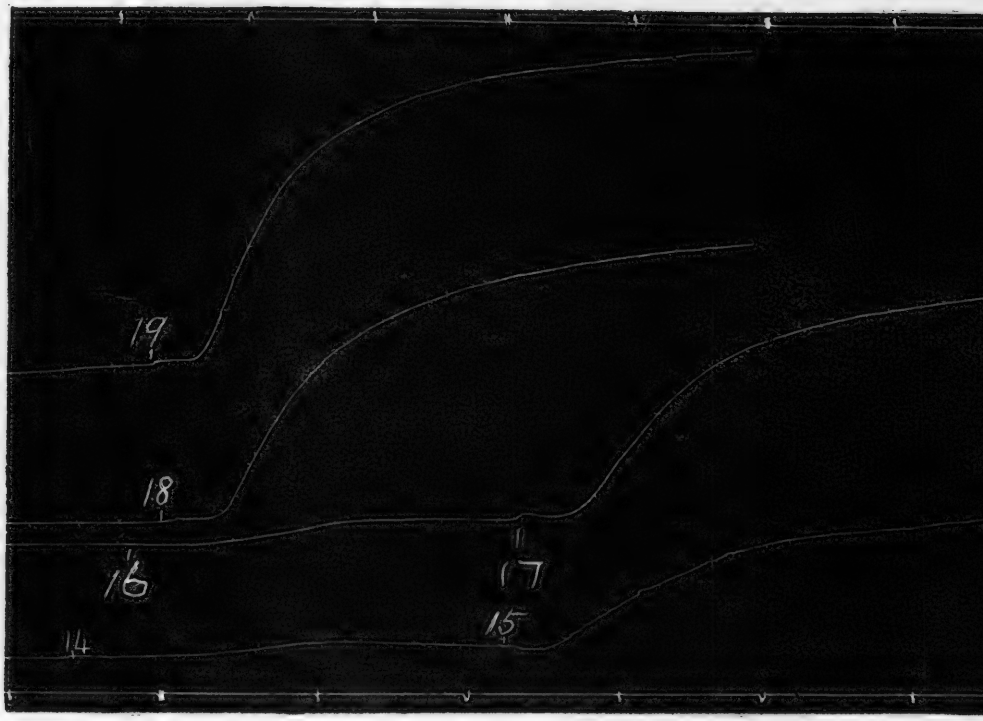


FIG. 13.

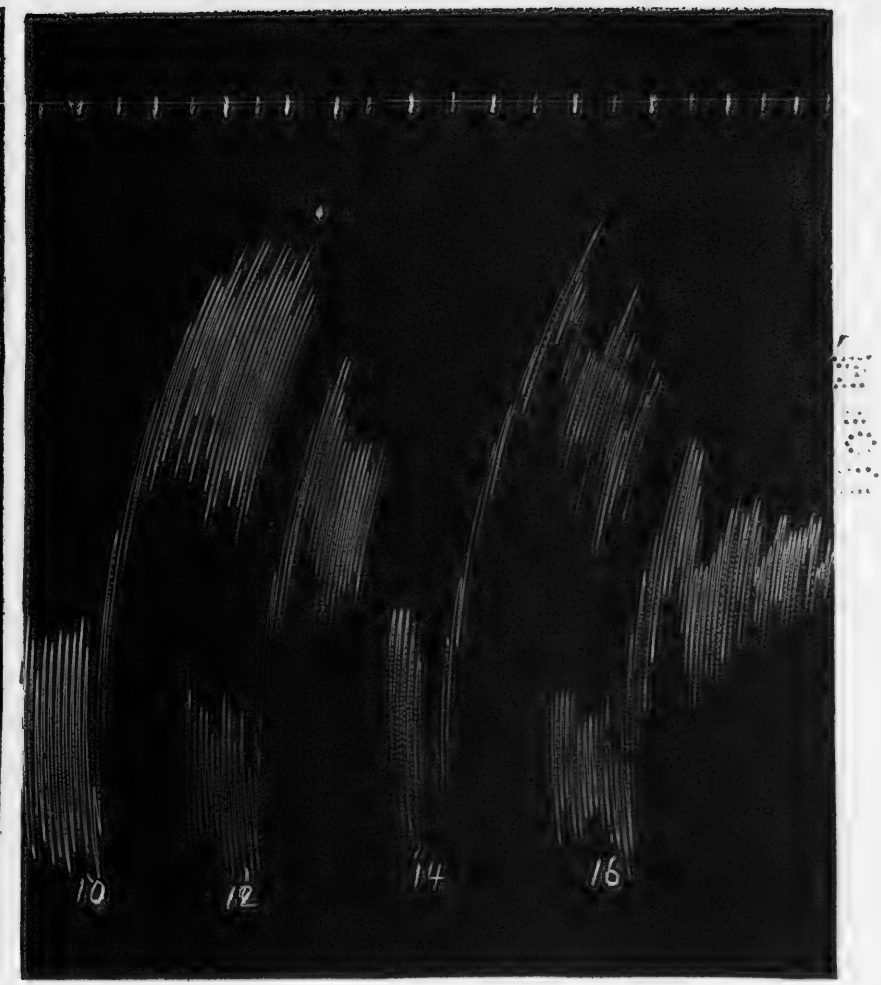
FIG. 14.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 17.

FIG. 15. Intestine. Rabbit hirudin material of Sept. 26. At 2 hirudin serum, at 4 hirudin plasma, at 6 hirudin serum replaced Ringer. At 10 hirudin serum diluted with two volumes of Ringer, at 12 hirudin serum diluted with five volumes of Ringer, at 14 hirudin plasma diluted with two volumes of Ringer, and at 16 hirudin plasma diluted with five volumes of Ringer replaced Ringer. Weight of intestine preparation, 0.37 gm.

Fi



15.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 18.

FIG. 16. Intestine. Hirudin material of Sept. 12. At 6 hirudin serum, and at 8 hirudin plasma replaced Ringer. At 10 Ringer was replaced by serum from the dog's clot diluted with its own volume of Ringer. The magnification of the movement by the lever was 2½ times less than that usually employed. Weight of intestine preparation, 0.40 gm.

FIG. 17. Intestine. Hirudin material of Aug. 29. At 2 hirudin plasma, at 4 hirudin serum, and at 6 hirudin plasma replaced Ringer. The segment was freshly prepared and observation 2 was the first one made on it. Weight of intestine preparation, 0.15 gm. It was much shorter than usual.

FIG. 18. Intestine. Rabbit hirudin material of Sept. 26. At 33 hirudin serum, at 35 the corresponding plasma, and at 37 the hirudin serum replaced Ringer. The intestinal segment was freshly prepared, and observation 33 was the first one made on it. Its weight was 0.34 gm.

FIG. 19. Intestine. Hirudin material of Oct. 2. At 2 hirudin serum, at 4 hirudin plasma, at 7 hirudin plasma, and at 12 hirudin serum replaced Ringer. Observation 2 was the first observation on this segment.

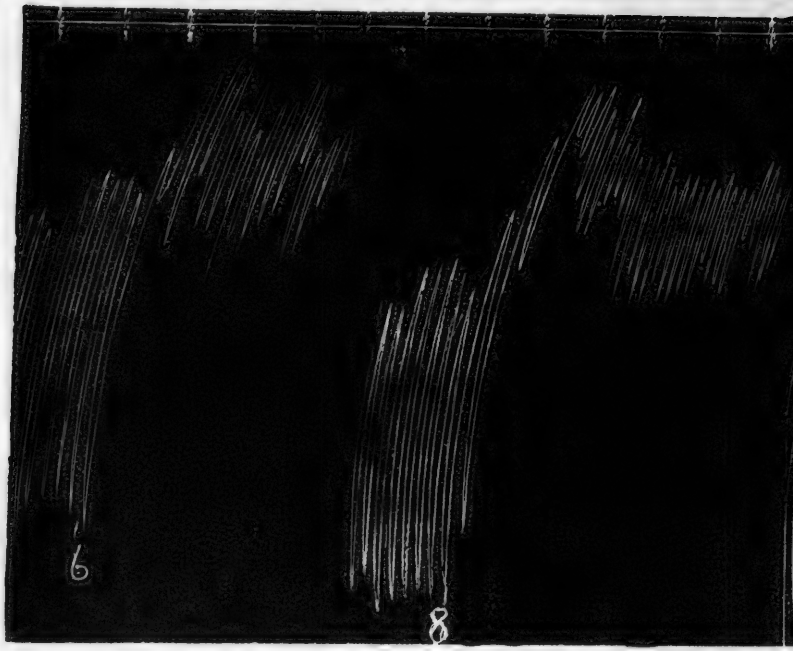


FIG. 16.

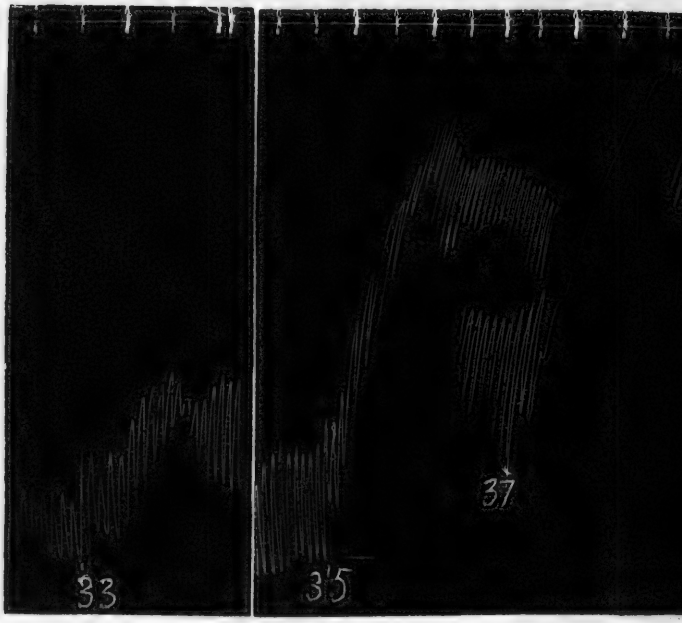
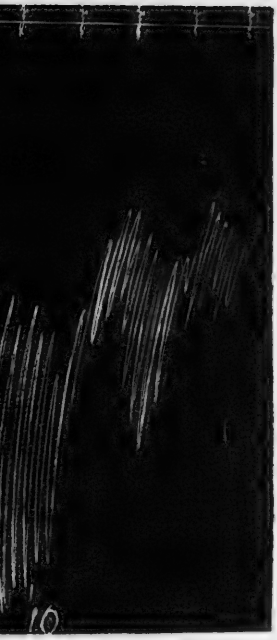


FIG. 18.



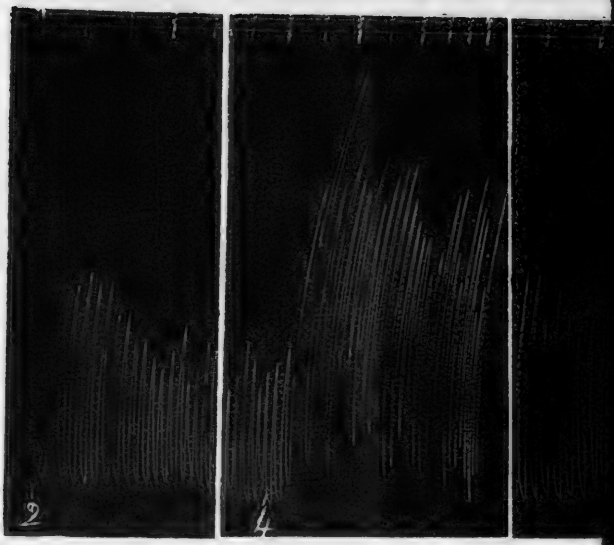


FIG. 17.

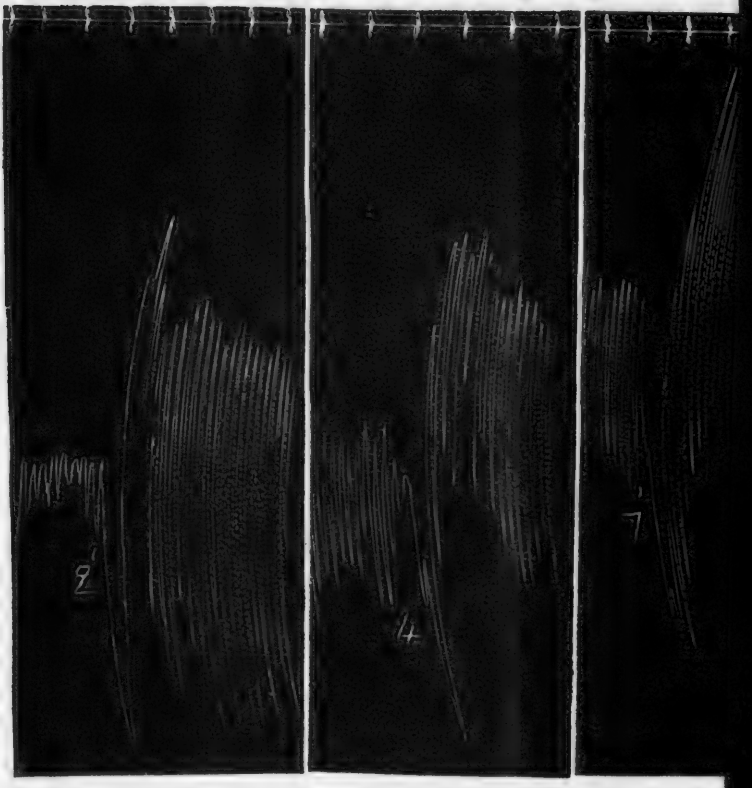


FIG. 19.

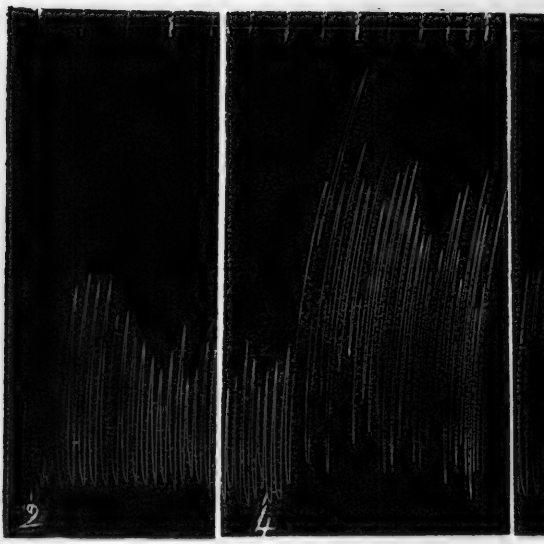


FIG. 17.

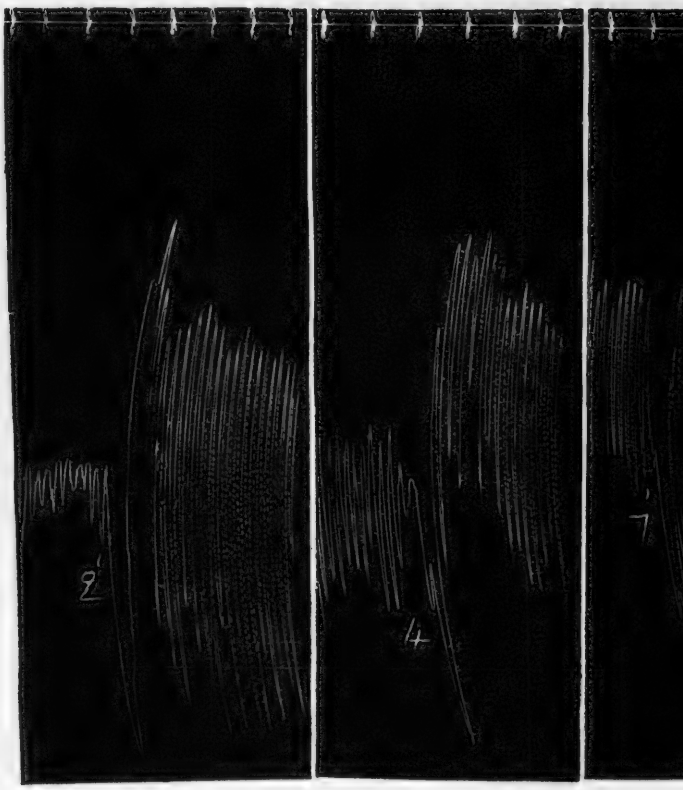
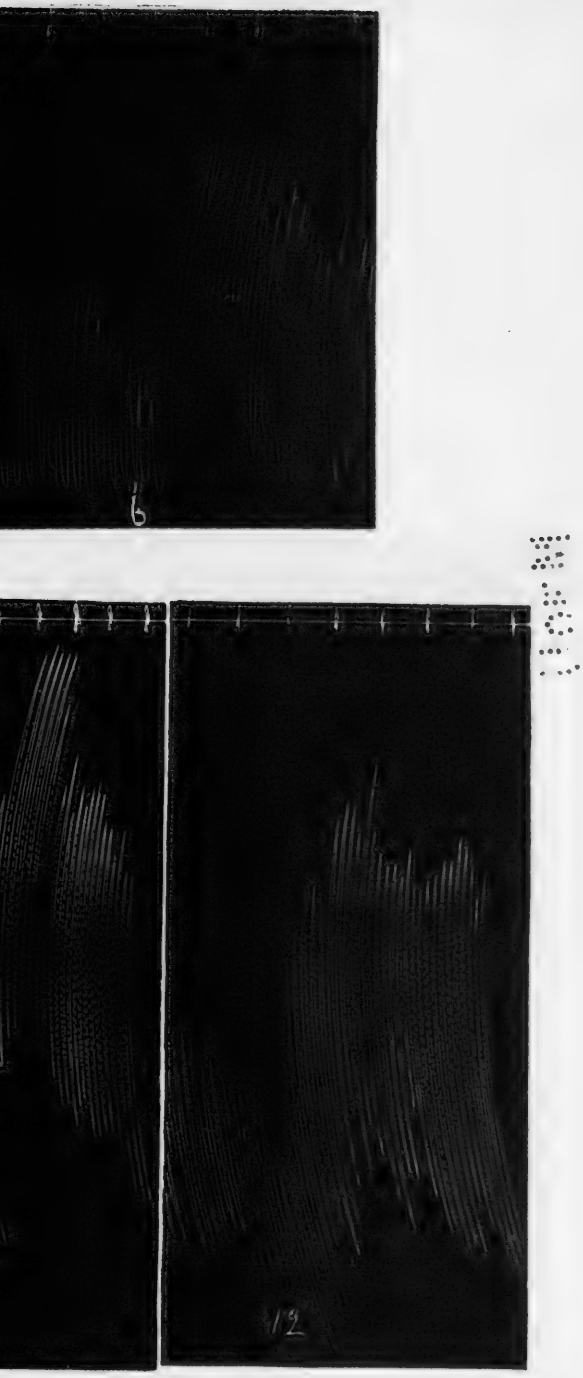


FIG. 19.



(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 19.

FIG. 20. Uterus. Rabbit hirudin material of Sept. 26. At 39 hirudin plasma, at 41 hirudin serum replaced Ringer. At 47 hirudin serum diluted with five volumes of Ringer, at 49 hirudin plasma diluted with five volumes of Ringer replaced Ringer. Weight of uterus preparation, 0.52 gm.

FIG. 21. Uterus. Dog hirudin material of Sept. 12. At 26 hirudin serum, and at 28 hirudin plasma replaced Ringer. At 30 Ringer solution was replaced by serum (from the dog's clot) diluted with its own volume of Ringer. The magnification of the lever was 2½ times less than that generally employed. Weight of uterus preparation, 0.74 gm.

FIG. 22. Uterus. Dog hirudin material of Aug. 29. At 24 hirudin plasma, at 26 the corresponding hirudin serum, and at 28 hirudin plasma replaced Ringer. The magnification was about half that usually employed. Weight of uterus preparation, 1 gm.

FIG. 23. The same material, the same uterus preparation, and the same magnification of the lever as those used for figure 22. At 30 the hirudin solution in Ringer diluted with two volumes of Ringer replaced Ringer. At 32 serum from dog defibrinated blood, at 36 adrenalin 1 : 1,000,000, and at 42 adrenalin 1 : 100,000 in Ringer replaced the Ringer solution.

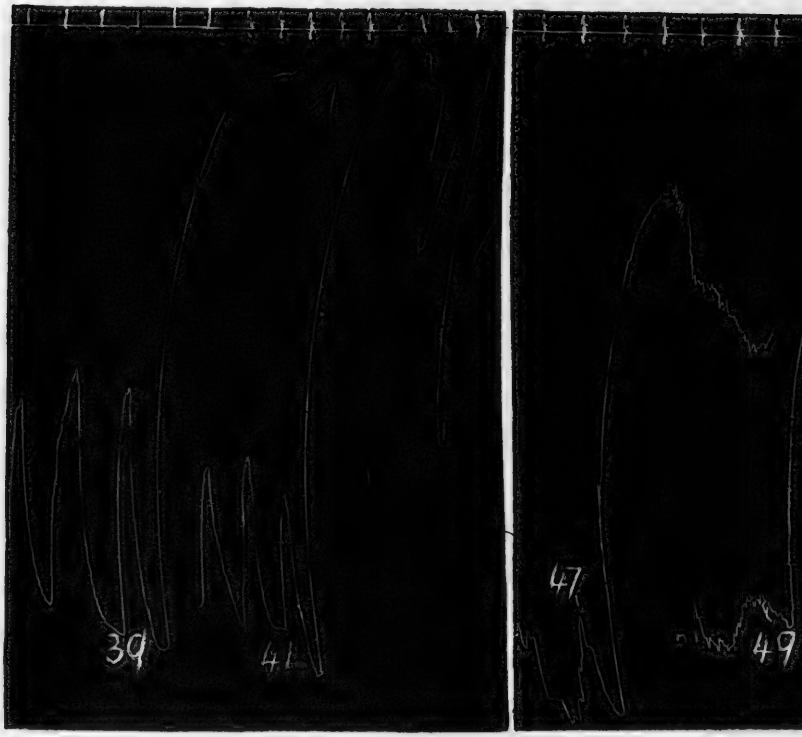


FIG. 20.

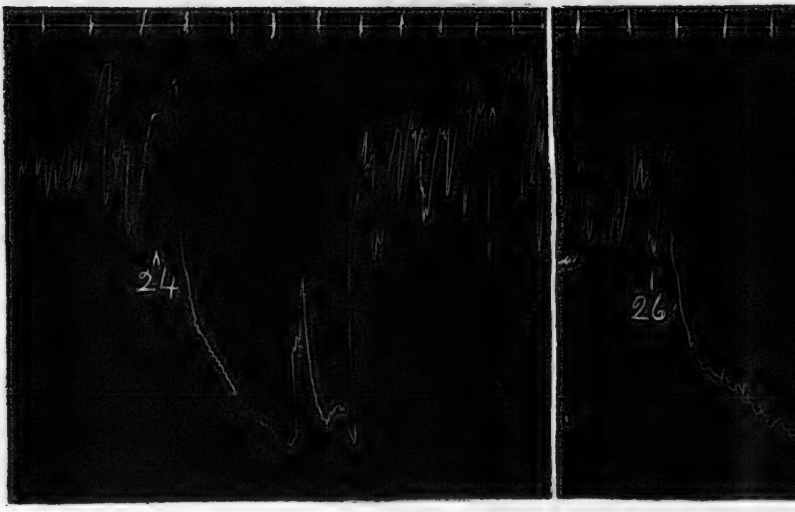


FIG. 21.

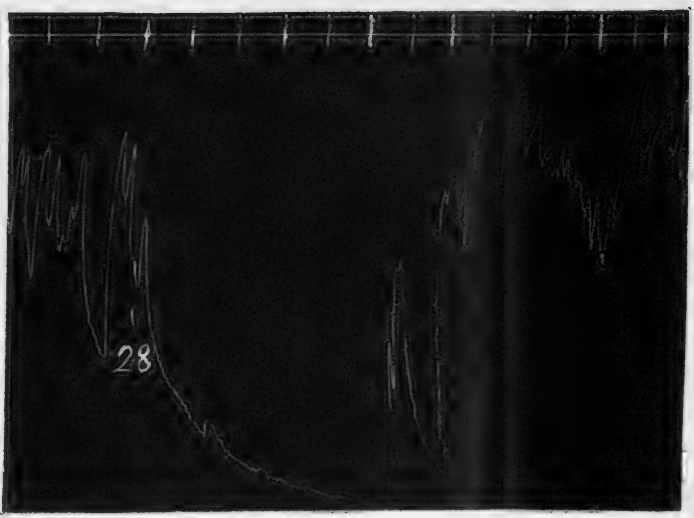


FIG. 22.

FIG. 21.

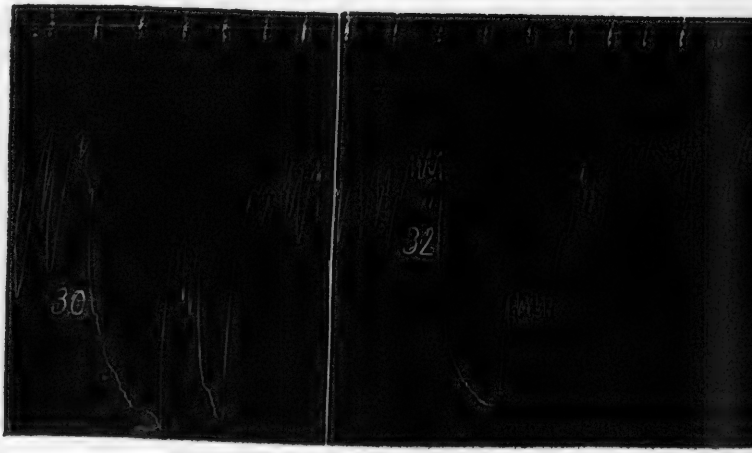


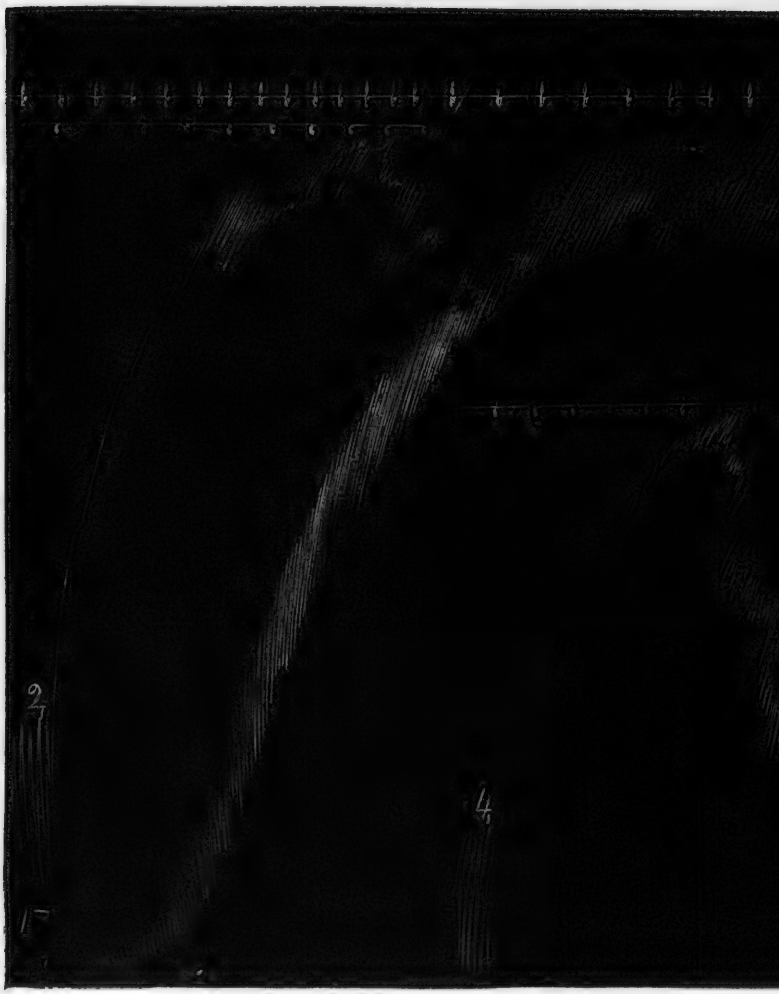
FIG.

23.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 20.

FIG. 24. Intestine. Extracts and modifications of sheep serum of July 25. At 2 the serum, at 4 A(3) (a fraction prepared with alcohol), at 9 the serum diluted with four volumes of Ringer, and at 11 H(6) replaced Ringer. At 15 with a fresh intestine preparation Ringer was replaced by H(5). The point went above the drum. At 17 the serum diluted with four volumes of Ringer replaced Ringer when the segment was beating feebly and the tone was very small. At 18 the dilute serum was itself replaced by H(6) and this at 19 by H(5). The writing point went far above the drum and then fell abruptly, the contractions disappearing. At 20 H(5) was replaced by H(6) and the curve soon rose again.



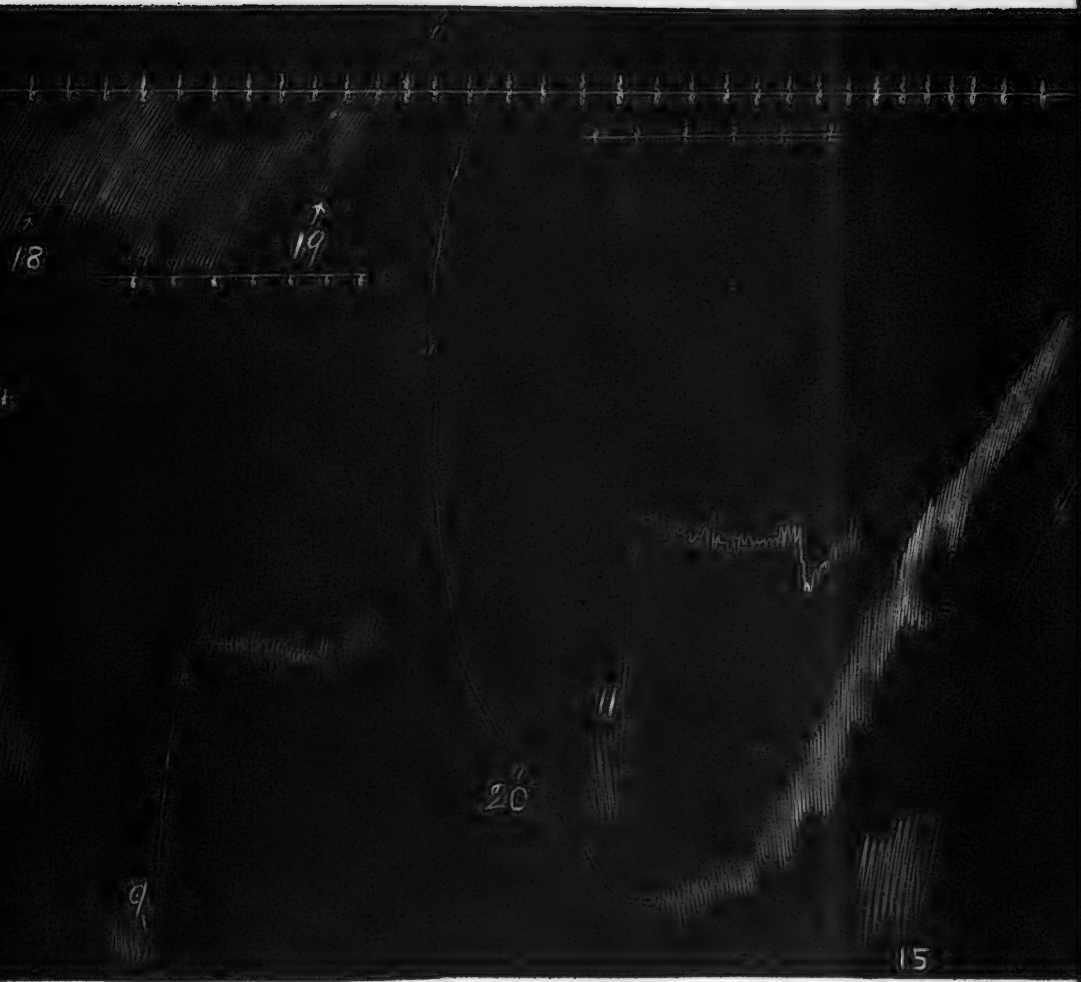


FIG. 24.

(Stewart and Zucker: Comparison o

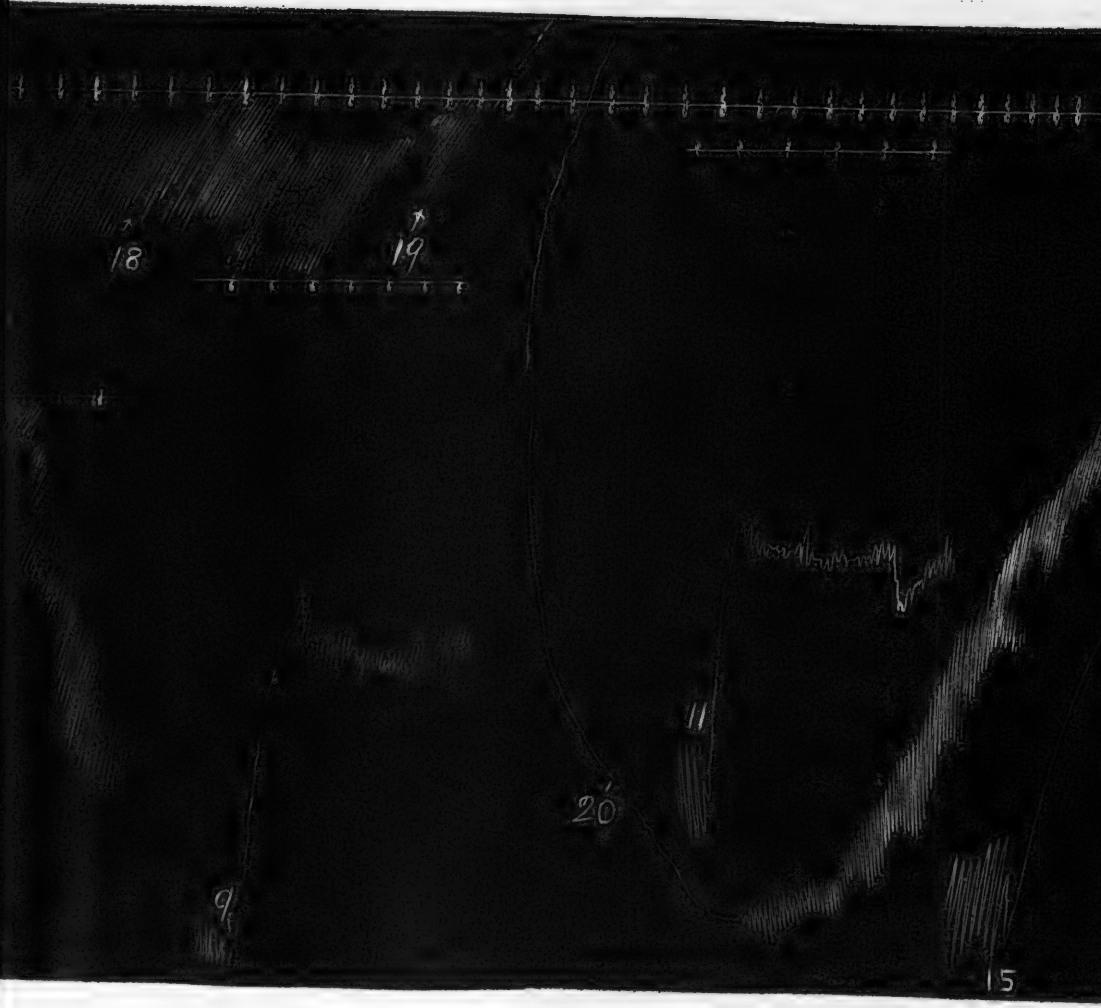


FIG. 24.

(Stewart and Zucker: Comp

PLATE 20.

rison of Action of Plasma and Serum.)

PLATE 21.

FIG. 25. Uterus. Action of the same material used in figure 24. At 14 serum replaced Ringer. At 16 A(3) replaced Ringer. The writing point went above the drum. At 24 Ringer was replaced by H(6) and at 31 by H(5). At 32 a little more of H(5) was added to replace that carried away by the oxygen stream.

FIG. 26. Intestine. At 2 egg white diluted with nine volumes of Ringer, at 13 egg white diluted with two volumes of Ringer, and at 27 undiluted egg white (strained) replaced Ringer. At 29 serum from the rabbit's own defibrinated blood, at 31 egg white diluted with one volume of Ringer, and at 33 rabbit serum diluted with one volume of Ringer replaced the Ringer solution. Weight of intestine preparation, which was shorter than usual, 0.25 gm.

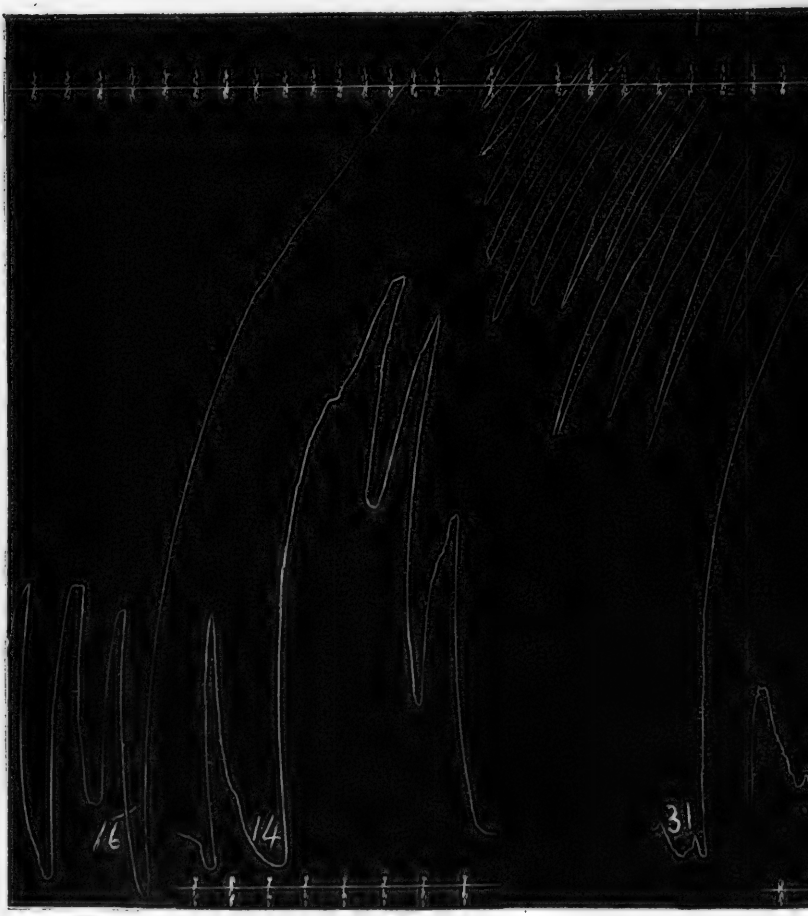
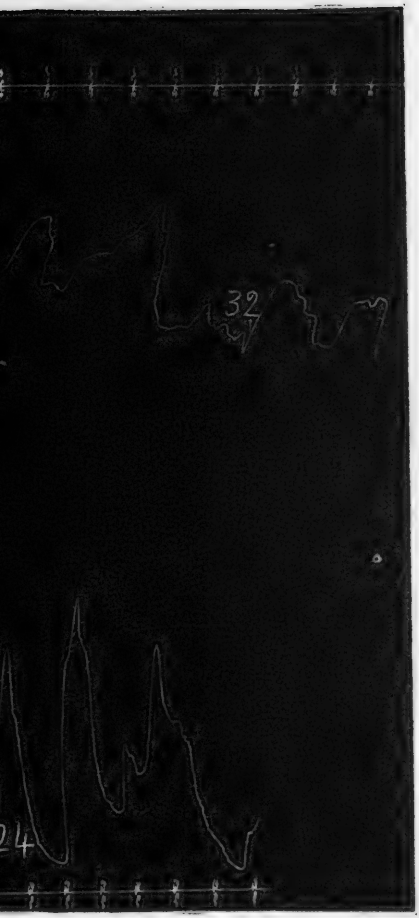


FIG. 25.



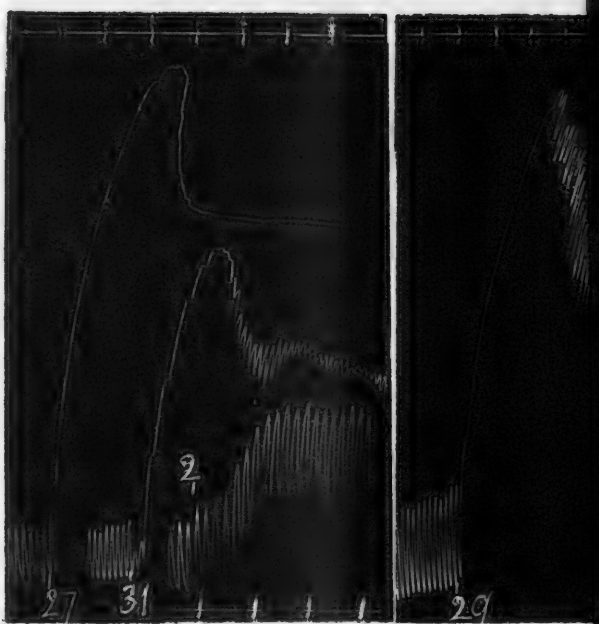
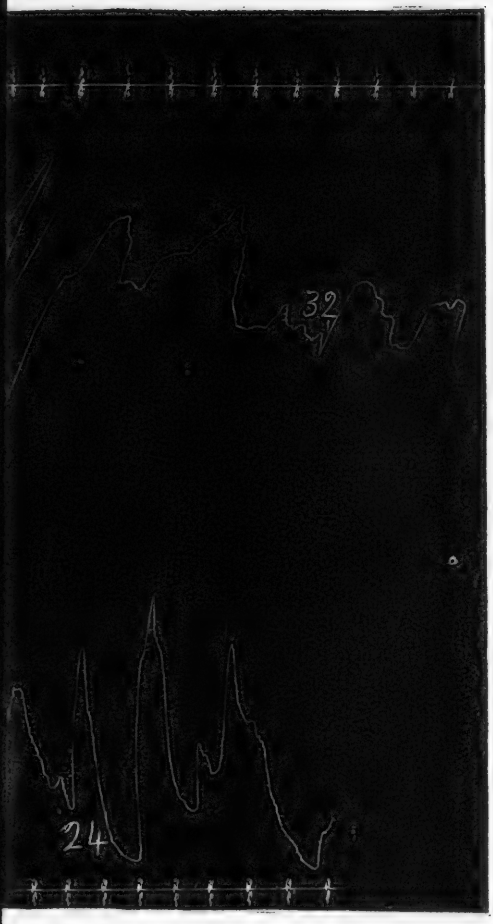


FIG. 26.

(Stewart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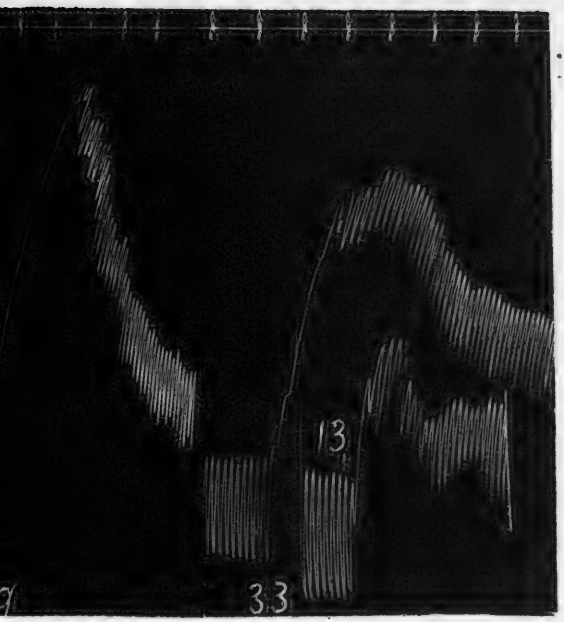

FIG. 26.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

conceivable that the alterations in the cells which are connected with the development of this property may even be wholly or partially independent of the cell changes concerned in coagulation. In this case it might be possible to obtain blood which would clot without developing the pressor property.

The main results which seem to follow from our observations may be thus summarized:

1. The substance, or property, developed in shed blood by which it causes constriction of artery rings, is not developed, or at least not mainly developed, in connection with the actual change of fibrinogen to fibrin, since the constrictor action of different plasmas differs greatly, while the absence of coagulation is common to all.

2. The development of the constrictor substance, or property, is associated with changes undergone probably by formed elements of the blood when it is shed. These changes may be identical with the alterations, or with some of the alterations, preliminary to clotting. It is possible, however, that there may be changes connected with the development of the pressor property which, although concomitant with the liberation of the substances concerned in the production of thrombin, are yet quite independent of the clotting process. Our observations do not enable us to decide definitely between these possibilities.

3. The constrictor substance, or property, developed in shed blood acts especially on blood vessels and does not equally affect the other organs examined. This follows from the fact that a plasma and serum which differ markedly in their action on the blood vessels may have practically the same action on the intestine or uterus segments, an action which must therefore reside mainly, at least, in the original plasma itself.

4. The indication that the serum acts especially on blood vessels increases the interest of the suggestion that this action may play an important part in the prompt sealing of wounded vessels in addition to the mechanical effect of the clot, or by coming into operation before the clot has fully formed.

THE ACTION OF HYDROCELE FLUID AND CERTAIN OTHER PATHOLOGICAL LIQUIDS ON SOME OF THE OBJECTS USED IN BIOLOGICAL TESTS FOR EPINEPHRIN.*

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(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland.)

Plates 22 to 27.

The question whether the tone-increasing action of serum on intestine and uterus segments is due altogether, or mainly, to the same property of the serum which causes it to constrict artery rings and to slow the stream through the frog perfusion preparation of Laewen[1] was tested by comparing the effect of hydrocele fluid on the four preparations. The typical hydrocele fluid is a transudate which, although containing fibrinogen, does not clot spontaneously, or at least not readily, but can be made to clot by the addition of thrombin or such liquids as blood serum which contain thrombin. Its poverty in thrombin is related to its poverty in formed elements. If the development of the property in shed blood by which it causes constriction of blood vessels is connected with changes in the formed elements, this property should be absent or very slightly marked in hydrocele fluid. If in spite of a negative result with blood vessels hydrocele fluid were found to cause an action on intestine and uterus segments comparable in magnitude to that of serum, the conclusion would be decidedly strengthened that the action of serum on these latter objects is different in nature from its action on the blood vessel preparation. In the following experiment these expectations were realized.

Hydrocele fluid was obtained with such precautions as insured the complete absence of blood. The fluid was the typical non-inflammatory hydrocele fluid, perfectly clear and yellowish in color, and it

* Received for publication, November 2, 1912.

[1] Stewart, G. N., and Zucker, T. F., *Jour. Exper. Med.*, 1913, xvii, 152.

remained free from the slightest clot for the whole period (over a week) during which it was kept in the ice chest. It was put on ice a very short time after being drawn. The total proteins were determined by precipitation with excess of alcohol.[2] The determination was made in duplicate on two samples of fifty cubic centimeters each. One yielded 2.37 grams, the other 2.38 grams; the protein content of 100 cubic centimeters was, therefore, 4.75 grams.

RESULTS.

Artery Rings.—Hydrocele fluid caused no noticeable effect when it replaced Ringer solution (figure 1, observation 8), while serum from a patient in diabetic coma replacing the hydrocele fluid (observation 9) caused a prompt and large constriction.

Frog Perfusion Preparation.—The slowing produced by the hydrocele fluid was slight and transient in comparison with that caused by dog serum, and the original outflow was quickly and completely restored (figure 2). The initial elevation on the curve is simply due to the mechanical increase of pressure caused by the injection.

Rabbit Intestine Preparation.—The increase of tone caused by the hydrocele fluid when it replaced Ringer solution was not far from equal to that caused by serum from the rabbit's own blood (figure 3, observations 2 and 6). This is shown even more strikingly in figure 4, where at 8 rabbit serum replaced Ringer solution and was itself replaced at 9 by hydrocele fluid. Instead of a fall, as would have been the case had the tone-increasing power of the hydrocele fluid been decidedly inferior to that of the serum, a sharp rise is seen at 9 due probably merely to the renewal of the liquid, and the curve then soon falls to the same height as before 9. When the observations were made in the reverse order, an almost identical curve was obtained. At 11, figure 4, Ringer solution was replaced by hydrocele fluid and the fluid at 12 by rabbit serum. The same transient rise occurred at 12 followed by a fall to the level which was being maintained before 12, indicating that the hydrocele fluid and serum were about equally potent in maintaining the increased tone.

That the actual change of fibrinogen into fibrin has little, if any-

[2] Hoppe-Seyler, F., Handbuch der physiologisch- und pathologisch-chemischen Analyse, 8th edition, Berlin, 1909, 662.

thing, to do with the tone-increasing property of shed blood for the intestine segments is illustrated by observations on a pleuritic exudate obtained from the right pleural cavity of J. S., a man aged twenty-two years. He had been ill for three weeks and complained of pain in the right side. The fluid was obtained free from blood and soon clotted into a jelly so that it could not be poured out of the bottle. The liquid from this clot was compared in a series of observations on the rabbit intestine segment with blood serum from the same rabbit. In figure 5 at 8 Ringer was replaced by the pleural liquid, and at 10 Ringer was replaced by rabbit serum. The increase of tone in 10 was distinctly greater than in 8. This result was obtained repeatedly on the same segment. Thus hydrocele fluid, in which no fibrin had been formed, had a tone-increasing power on the intestine equal to that exerted by rabbit serum; while the pleural liquid, after the formation of much fibrin, was inferior to rabbit serum in this regard. The liquid was richer in protein than the hydrocele fluid, containing 3.081 and 3.069 grams in two portions of fifty cubic centimeters, *i. e.*, 6.15 grams in 100 cubic centimeters. The pleuritic exudate probably was, or had been, richer in formed elements than the hydrocele fluid, so that if cell decomposition or changes of permeability in the cells are responsible for the tone-increasing property as regards the intestine that property ought to have been more strongly developed in the pleuritic fluid.

That the change of fibrinogen into fibrin is not necessarily associated with the development of the pressor property of shed blood for artery rings, any more than with its tone-increasing power for intestine and uterus, was demonstrated by observations on fluid from a cyst of the suprarenal gland. This fluid was unclotted when obtained, but could be made to clot in the thermostat. The action of the liquid before and after clotting was compared on sheep artery rings and rabbit intestine and uterus segments (figures 6 to 9), and was found to be practically identical. This was not because the epinephrin which it contained caused an effect so great as to obscure any difference which might have existed. For it was shown that the concentration of epinephrin present, although considerable (probably somewhat less than 1:1,000,000), produced a much

smaller pressor effect on the rings and a much smaller increase of tone in the uterus segment than did the cyst liquid. The extremely prompt rise in the case of the uterus preparation, which is by no means peculiar to the action of this liquid, but is characteristic of the reaction of the uterus segments to serum, precludes the possibility that the tone-increasing property of the liquid is developed after contact with the tissue. It is only a matter of seconds when the rise begins. Further, after emptying the apparatus no clot could be seen.

Incidentally we have here an example of a plasma (an unclotted serous liquid) which presented no advantages over the corresponding serum (the cyst liquid after coagulation) for epinephrin-testing.

Rabbit Uterus Preparation.—On the uterus preparation of the same rabbit, a young non-pregnant adult whose intestine had been used for testing the hydrocele fluid, the tone-increasing power of the hydrocele fluid was distinctly less than that of the rabbit serum, so that when the hydrocele fluid was caused to replace the serum at 19, figure 10, the tone of the preparation was decidedly diminished. When hydrocele fluid replaced Ringer solution the increase of tone was inferior to that occasioned by the rabbit serum. Adrenalin 1:5,000,000 caused an increase of tone after a slight preliminary inhibition, adrenalin 1:50,000 a marked increase of tone without preliminary inhibition. After being kept four days in the ice chest, during which time it remained perfectly clear and there was no sign of bacterial growth, the hydrocele fluid was again tested on the uterus segment from an adult non-pregnant rabbit (figure 11). When the hydrocele fluid replaced Ringer, a rise of tone (observation 38) was obtained of about the same magnitude as that caused by adrenalin 1:1,000,000 (observation 41) or of hirudin whole blood from a dog (observation 30). The rise occasioned by the hydrocele fluid in this observation appears to be much more transient than is really the case, since the drum was moving more slowly than in the other observations. For the sake of comparison, observation 28, showing the effect of hirudin defibrinated blood replacing Ringer solution, is also reproduced. Observation 32 shows the action of the undiluted dog serum, without hirudin, and observation 34 that of the serum diluted with its own

volume of Ringer. The hydrocele fluid produces a maximum increase of tone fully equal to that given by the diluted serum, although not quite so durable. It will be remarked that as regards its protein content it also resembles a somewhat diluted serum. This agrees very well with the view that the action of the hydrocele fluid on the intestine and uterus is mainly a general nutritive action of the same nature as that of the serum. If we conceive, on the other hand, the serum effect as being due to a special substance originally present in the blood plasma, it would be necessary to assume that this substance in passing into the hydrocele fluid was diluted in approximately the same degree as the plasma proteins.

With rings of sheep artery dog serum, without hirudin, and hirudin serum gave a very good rise. The dog hirudin plasma also caused a good rise, although not so great as that given by the sera.

The results of all the observations on the hydrocele fluid strengthened the conclusion that the constrictor action of serum for blood vessels is not a general action of the serum common to other serous liquids, but that it is due to a property developed in the shed blood which has a special relation to blood vessels.

Some observations were also made on ascitic fluid obtained twice, at an interval of seven weeks, from the same patient.

J. M., a man 50 years old, was admitted on July 6, 1912, to the City Hospital, suffering from cardiorenal disease (mainly renal) with a high systolic pressure (200 mm. of mercury), and general edema involving the face and the upper and lower limbs. The urine constantly contained albumen and casts. The left border of the cardiac dullness was two fingers' breadth outside the nipple line. On auscultation at the apex the first sound was impure, the second sound loud. In the aortic area the second sound was much accentuated and the pulmonary second was also accentuated. The abdomen was distended with free fluid.

On July 11, eight liters of liquid were removed from the abdomen, on August 19 more than seven liters, and on August 28, nearly two liters. The specimens were obtained quite free from blood. The action of the liquid obtained on July 11 and August 28 was determined on artery rings, and on segments of intestine and uterus. The protein in the fluid of July 11 was 0.637 gm. and 0.623 gm. in two portions of 50 c.c., *i. e.*, 1.26 gm. in 100 c.c. In the specimen of August 28 somewhat more protein was present, 1.055 and 1.029 gm. in two portions of 50 c.c., *i. e.*, 2.084 gm. in 100 c.c. Neither specimen showed any trace of clotting, although kept for several days.

With the artery rings no rise whatever was caused (figure 1, observation 10) whereas a serum used for comparison produced a very good rise (observation 11).

On the intestine preparation the effect was an increase of tone accompanied by an increase in the size of the rhythmical contractions. The effect was qualitatively similar to that produced by serum from the same patient, as shown in figure 12, for which the material of July 11 was used. At 4 Ringer solution was replaced by the ascitic fluid, and at 6 by serum from the patient's clotted blood obtained at the same time. The tone-increasing effect of the undiluted serum was decidedly greater than that of the peritoneal liquid.

Observations 8 to 14 (figure 12) were made with the object of determining in what dilution the serum effect would be approximately equal to that of the liquid. At 8 Ringer solution was replaced by serum diluted with two volumes of Ringer, at 12 by serum diluted with five volumes of Ringer, at 14 by ascitic fluid. The effect produced by the fluid was not very different, as regards increase of tone, from that produced by the serum diluted with five volumes of Ringer, and was superior, as regards increase in the size of the beats. Although no great stress should be laid on the coincidence, it may be noted that here also the pathological serous liquid exerted an action which, as compared with that of the serum, was approximately proportional to its content of protein.

On the uterus preparation (figure 13) from an adult non-pregnant rabbit the ascitic fluid (of July 11) produced a good increase of tone followed by complete relaxation (observation 21). The relaxation was especially evident when the fluid was added at a time when the uterus segment possessed a high initial tone (observation 38). At 39 it was particularly observed that relaxation was complete. The relaxation following the increase of tone was equally complete in observation 21 where the initial tone was low. The contractions ceased entirely during the relaxation.

On the uterus preparation from another rabbit (non-pregnant, small, and immature) the same phenomenon of relaxation with cessation of contraction was observed (figure 14, observation 24) succeeding a preliminary increase of tone. On the same segment the serum of the patient caused a good rise of tone which was sustained (observation 22).

The specimen of ascitic fluid obtained from the same patient on

August 28 caused a fair increase of tone in a rabbit intestine segment, although distinctly less than that produced by dog serum diluted with five volumes of Ringer solution.

On the uterus preparation from an adult non-pregnant rabbit the same inhibition succeeding the increase of tone which was observed with the other specimen of the fluid from this patient was noted. We are not in a position to state what the cause of this inhibition was. The other transudate examined (hydrocele fluid) also exhibited this effect, although to a smaller extent. It is a phenomenon which we have not observed with blood serum or plasma and is probably due to some constituent of the pathological liquids not present, or present in much smaller amount in blood. However this may be, the uterus observations again illustrate the point on which we have more than once insisted elsewhere, that we cannot postulate without experiment a similar action of a given serous liquid on different test objects any more than we can postulate a similar action of different serous liquids on one and the same test object. Each liquid must be investigated separately for each test object.

This point is well illustrated by the tracings reproduced in figures 15 and 16 in which the action of blood serum from a fatal case of diabetic coma is compared with that of dog serum (of September 12). In figure 15 with a rabbit intestine preparation, Ringer solution was replaced at 14 by the dog serum and at 16 by the diabetic serum. In figure 16 with the uterus preparation from the same rabbit (a non-pregnant adult), Ringer solution was replaced at 36 by the diabetic serum and at 38 by the same dog serum as was used in figure 15. The increase of tone in the uterus segment caused by the diabetic serum was much greater than that produced by the dog serum, whereas the dog serum occasioned a greater increase of tone in the intestine segment than the diabetic serum. On the artery rings the diabetic serum caused an unusually prompt and large effect.

The diabetic serum was obtained from a young woman twenty years old, a telephone operator. She collapsed from overwork and was treated by her physician for nervous breakdown for some weeks before she applied for treatment at Lakeside and Western Reserve University dispensary. On admission at the dispensary she showed tachycardia, exophthalmos, slight thyroid enlargement, and loss of weight. Sugar was present in the urine to the extent of 3 per cent.

EXPLANATION OF PLATES.

All tracings are to be read from left to right. Time is marked in half minutes. Unless otherwise mentioned the liquid to be tested was always caused to displace the Ringer solution in which the preparation was immersed. Two c.c. of the liquid to be tested were always added, which was sufficient to displace the Ringer solution. The weight of the preparations, after drying with blotting paper, is given to facilitate comparison of the mass of tissue acted on, with the amount of active substance.

PLATE 22.

FIG. 1. Sheep artery rings. At 8 hydrocele fluid replaced Ringer, at 9 serum from a patient in diabetic coma replaced the hydrocele fluid. The slight rise in the curve after 8 does not indicate a genuine constriction due to the hydrocele fluid. It was probably caused by accidental jarring. At 10 ascitic fluid from J. M. replaced Ringer, and was itself replaced at 11 by dog serum. The rise produced by the dog serum is more abrupt than was observed with any other serum except that from the case of diabetic coma.

FIG. 2. Frog perfusion preparation. Curves showing changes in outflow with hydrocele fluid and dog serum, each diluted with three volumes of Ringer solution. The number of drops per half minute is laid off along the vertical, and the time in half minutes along the horizontal axis. Each small division corresponds to a half minute. Twenty-one drops correspond to 1 c.c.

FIG. 3. Rabbit intestine. Weight of preparation, 0.27 gm. At 2 hydrocele fluid, at 4 serum from the rabbit's blood, and at 6 hydrocele fluid replaced Ringer.

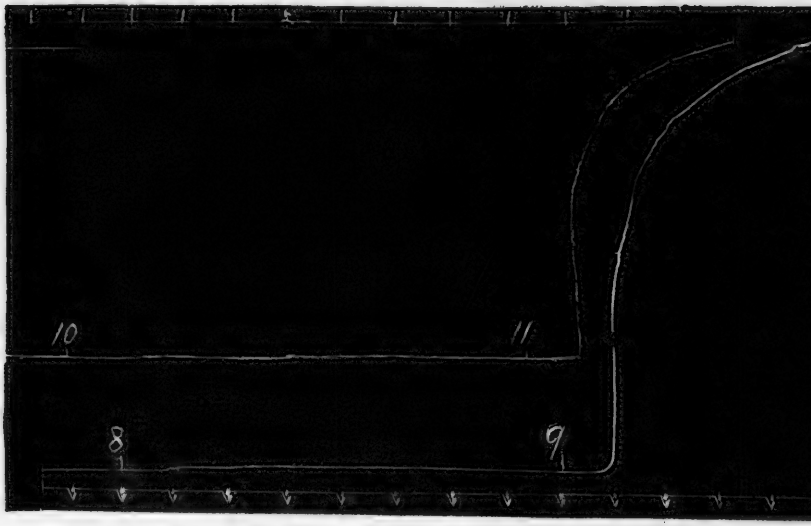


FIG. 1.

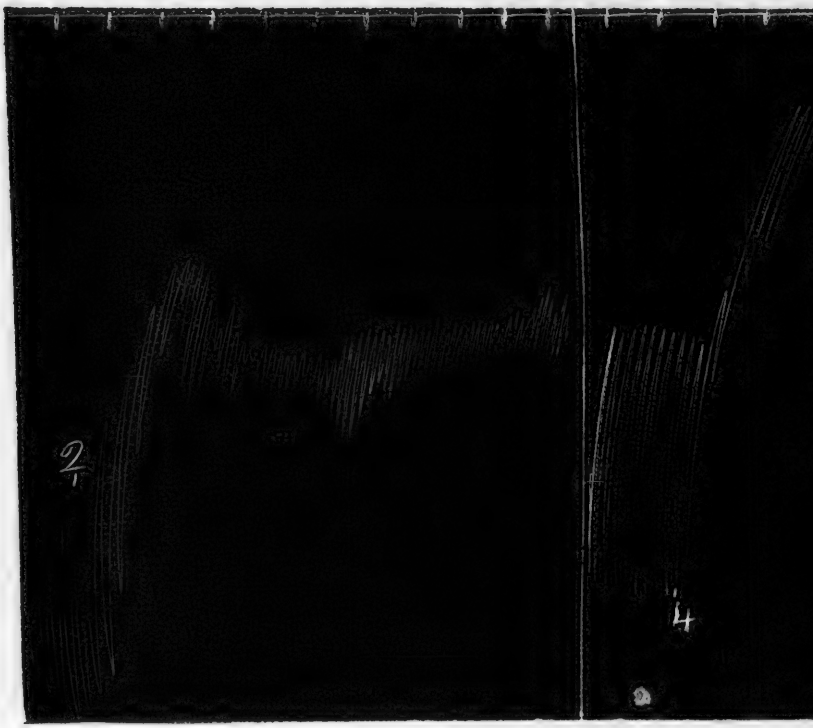


FIG. 3.

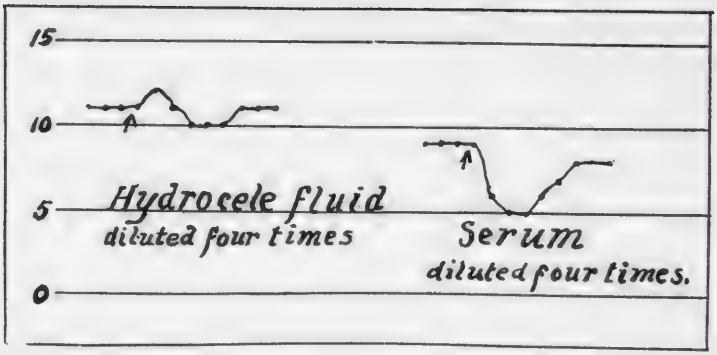
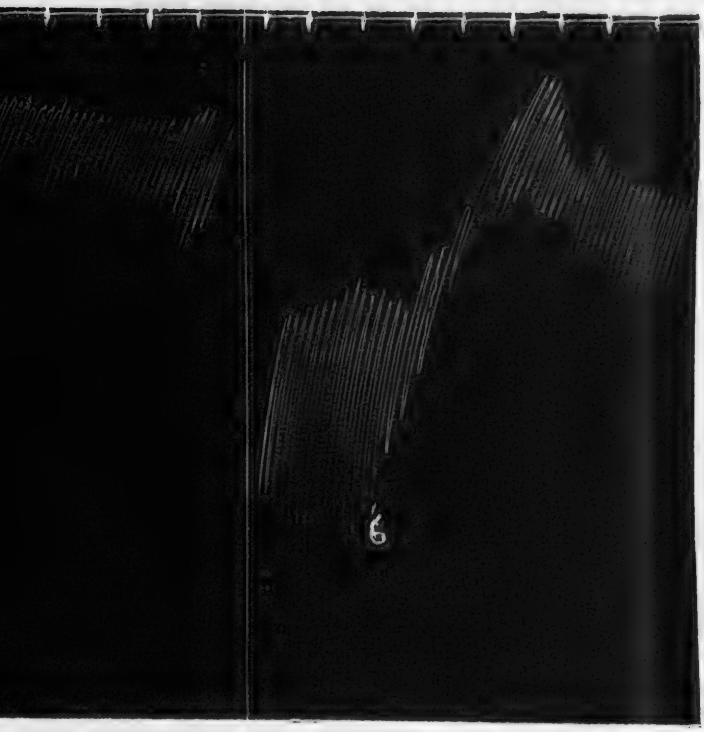


FIG. 2.



(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 23.

FIG. 4. The same intestine preparation as that used for figure 3. At 8 Ringer was replaced by the rabbit's own serum, and this at 9 by hydrocele fluid. At 11 Ringer was replaced by hydrocele fluid, and this at 12 by rabbit serum.

FIG. 5. Intestine. Weight of preparation, 0.34 gm. At 8 serum from clotted pleural fluid (from J. S.) replaced Ringer. At 10 rabbit serum replaced Ringer.

FIG. 6. Sheep artery rings. Fluid from cyst of suprarenal gland. At 3 Ringer was replaced by the fluid which had clotted at 4 by the unclotted fluid at 5 by adrenalin solution in Ringer, 1 : 50,000 (freshly opened).

FIG. 7. Intestine preparation. Weight of preparation, 0.30 gm. At 8 Ringer was replaced by unclotted adrenal cyst liquid, at 10 by cyst liquid after it had clotted.

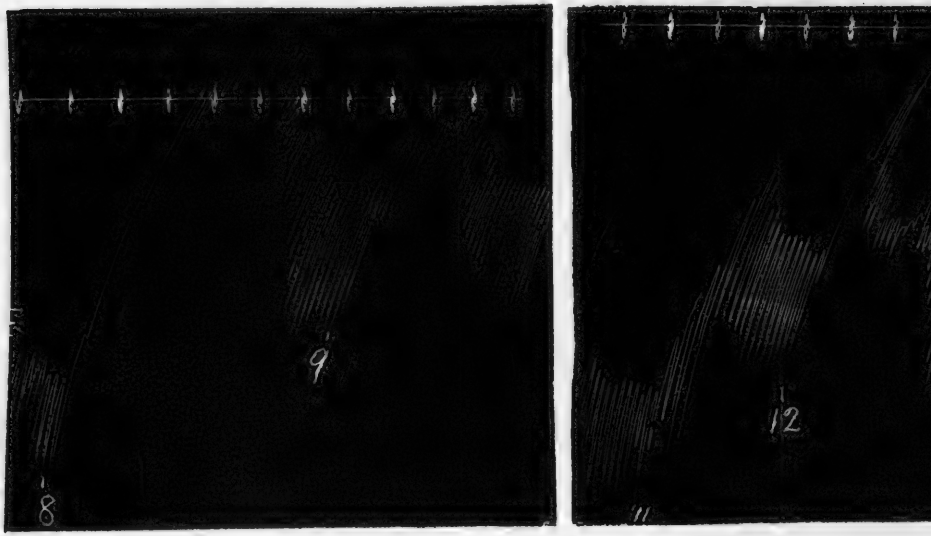


FIG. 4.

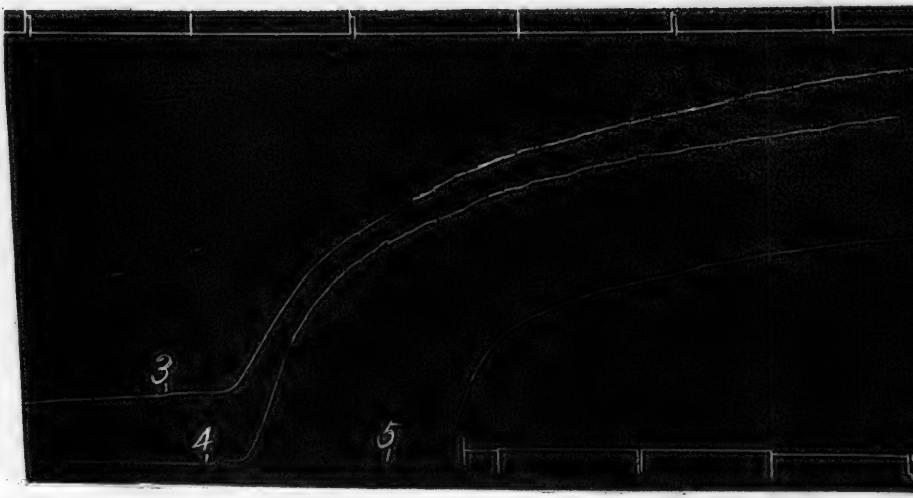


FIG. 6.

FIG. 5.

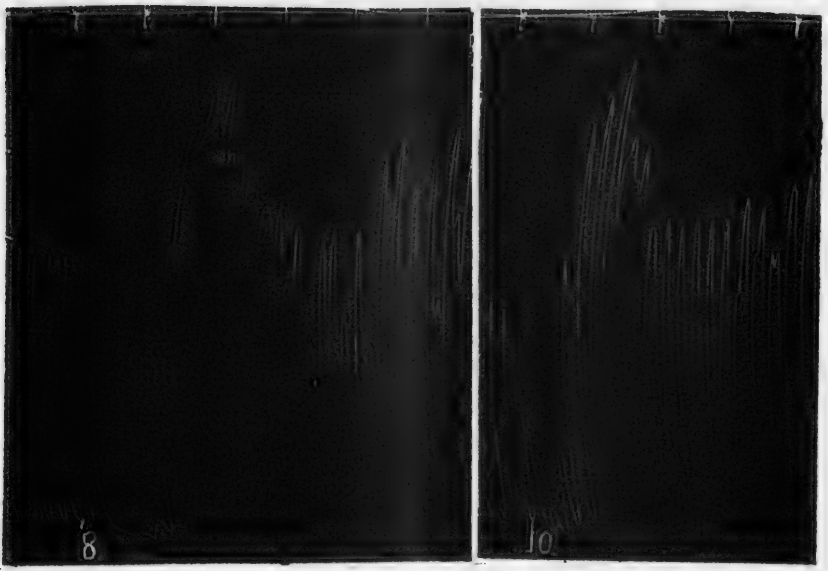


FIG. 7.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 24.

FIG. 8. Same intestine preparation as used in figure 7. At 12 Ringer was replaced by the unclotted cyst fluid, at 14 by the cyst fluid after clotting. At 16 sheep serum replaced Ringer and was itself replaced at 17 by the unclotted cyst fluid.

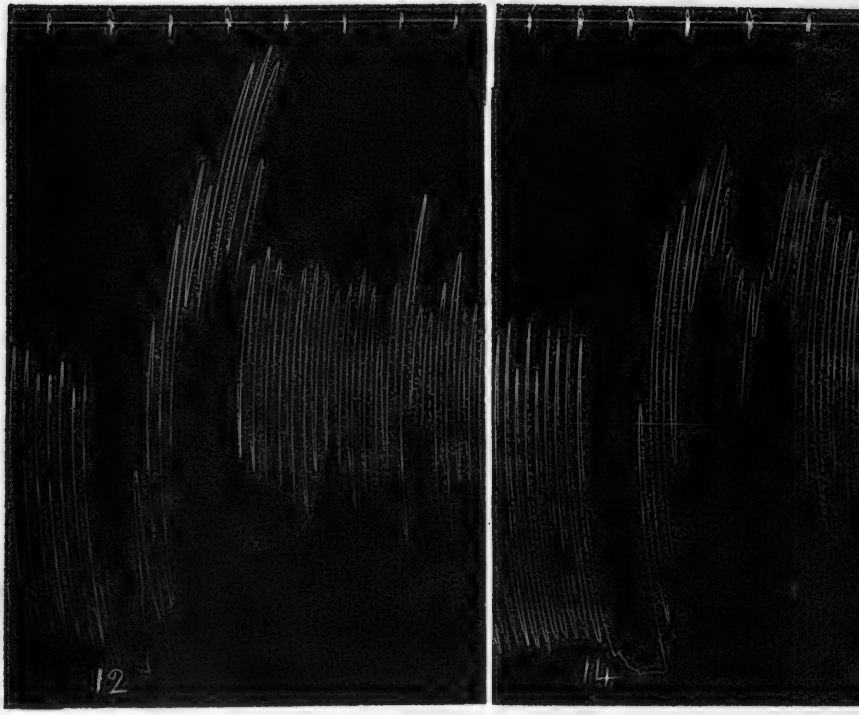
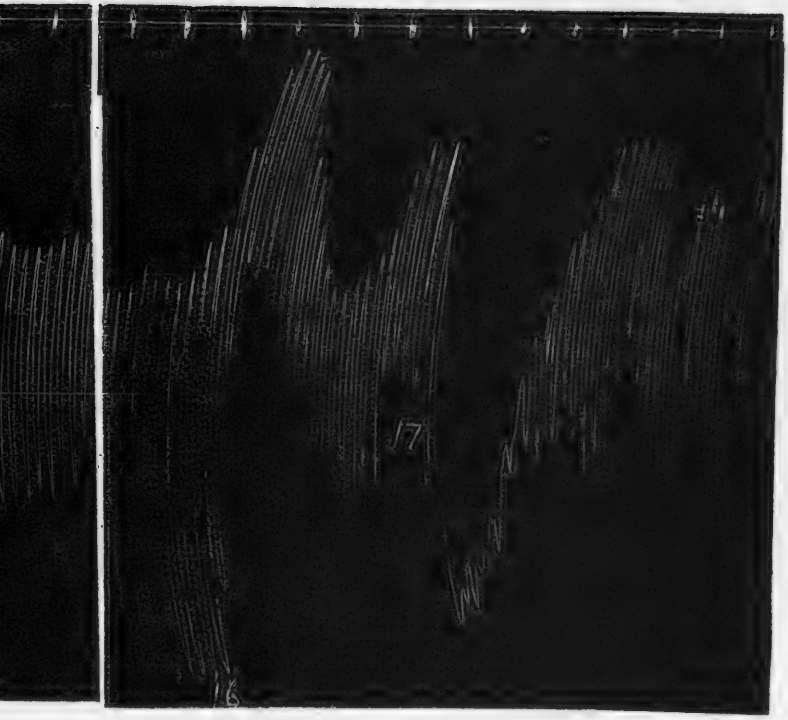


FIG. 8.



(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 25.

FIG. 9. Uterus preparation from an adult non-pregnant rabbit. Weight of preparation, 0.30 gm. At 28 Ringer was replaced by the unclotted cyst liquid, at 30 by the cyst liquid after it had clotted. The writing point went a little above the drum and then gradually came down. At 32 Ringer was again replaced by the unclotted cyst liquid.

FIG. 10. Uterus from a young non-pregnant rabbit. Weight of preparation, 0.17 gm. At 18 Ringer was replaced by rabbit serum and this at 19 by hydrocele fluid. At 20 additional hydrocele fluid was run in. The magnification was two thirds of that usually employed.

FIG. 11. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.71 gm. Magnification 2½ times less than that usually employed. Comparison of hydrocele fluid with material obtained from a dog on Sept. 10. At 28 Ringer was replaced by the dog's hirudin defibrinated blood, at 30 by the dog's whole hirudin blood, at 32 by undiluted serum from the dog's clot, at 34 by serum from the dog's clot diluted with its own volume of Ringer, at 38 by hydrocele fluid, and at 41 by adrenalin 1 : 1,000,000 in Ringer solution. The time trace above observation 28 shows that the drum was then moving much more slowly than during the other observations.

FIG. 9.

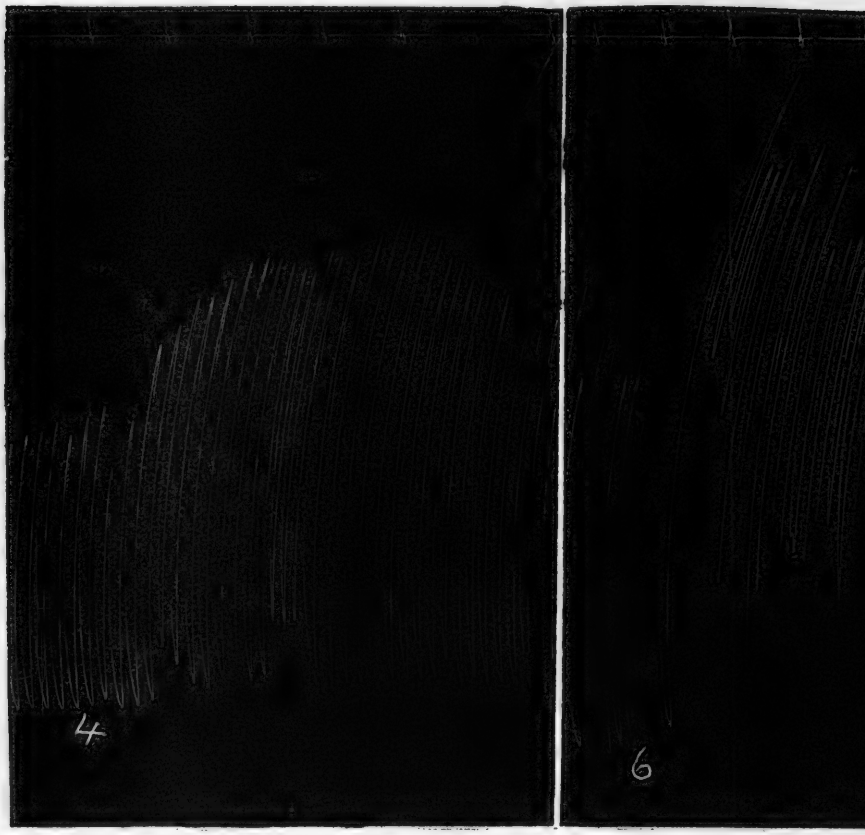
FIG. 11.

FIG. 10.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 26.

FIG. 12. Intestine. Weight of preparation, 0.25 gm. At 4 ascitic fluid from J. M., at 6 serum from J. M., at 8 his serum diluted with two volumes of Ringer, at 12 his serum diluted with five volumes of Ringer, and at 14 his ascitic fluid replaced Ringer.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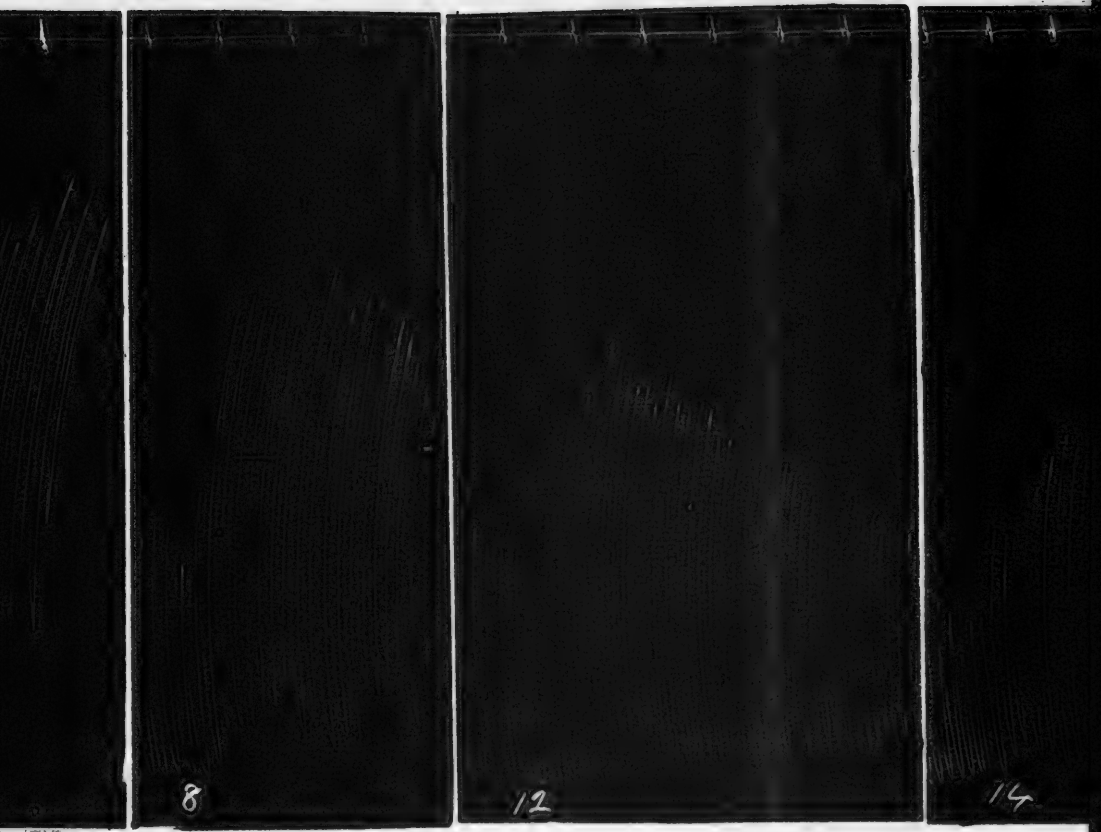

FIG. 12.

(Stewa

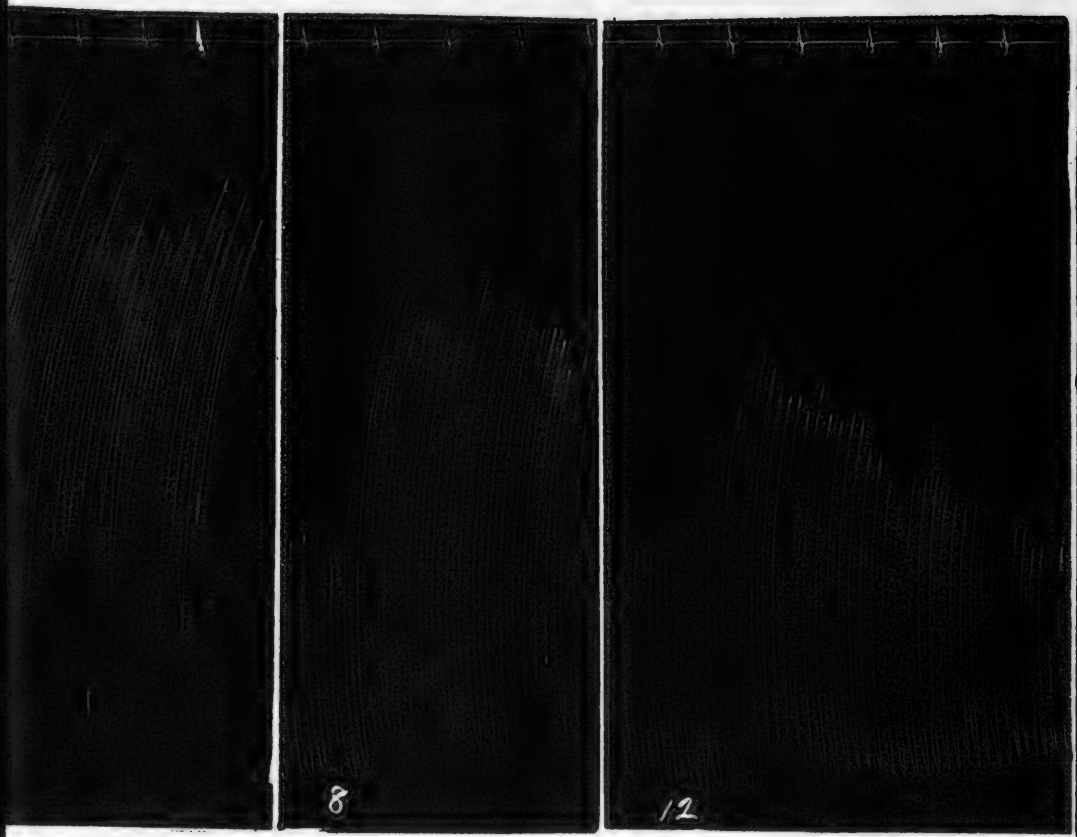

FIG. 12.

PLATE 26.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 27.

FIG. 13. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.55 gm. At 21 ascitic fluid from J. M. replaced Ringer. The initial tone of the uterus segment was small. At 38 the ascitic fluid replaced Ringer when the initial tone of the segment was high. At 39 the preparation was seen to be completely relaxed. The magnification was 2½ times less than that usually employed.

FIG. 14. Uterus preparation from a young non-pregnant rabbit. Weight of preparation, 0.45 gm. At 22 J. M.'s serum, diluted with five volumes of Ringer solution, and at 24 his ascitic liquid replaced Ringer.

FIG. 15. Intestine. Weight of preparation, 0.40 gm. At 14 serum from dog's clot, at 16 serum from a patient in diabetic coma replaced Ringer. The magnification was 2½ times less than that generally used.

FIG. 16. Uterus. Weight of preparation, 0.76 gm. At 36 serum from the patient in diabetic coma, and at 38 serum of the clot of the same dog that was used in figure 15 replaced Ringer. The magnification was the same as in figure 11.

FIG. 13.

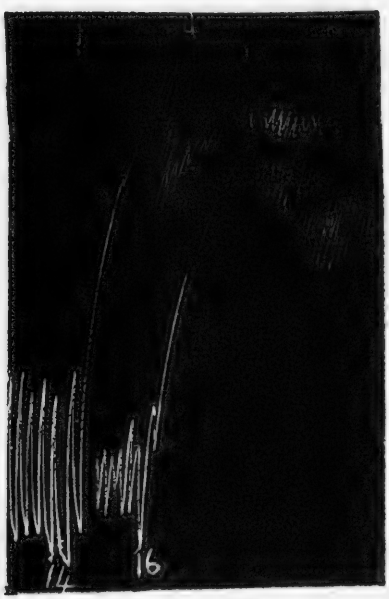

FIG. 15.

FIG. 16.
(Stewart and

FIG. 14.

(Zucker: Action of Hydrocele Fluid.)

The diagnosis made was Graves' Disease with glycosuria. She was put on a non-carbohydrate diet, and returned the following week slightly improved. There was still some sugar in the urine. The same treatment was continued. She returned the next week and said she was worse and entered the hospital, where she quickly developed well marked coma with a distinct odor of acetone in the breath. The non-carbohydrate diet was cancelled, but the coma progressively deepened and she died about twelve hours later without regaining consciousness. Ten hours before death 100 c.c. of blood were removed preparatory to the giving of sodium bicarbonate intravenously. This was the specimen of blood tested.

We are indebted to the staffs of Lakeside Hospital and the City Hospital, especially to Dr. Bucher of the City Hospital, for the material investigated.

STUDIES OF THE DEGENERATION AND REGENERATION OF AXIS CYLINDERS IN VITRO.*

By RAGNVALD INGEBRIGTSEN, M.D.

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PLATES 28 TO 40.

The experiments of Harrison,[1] who cultivated the medullary cord of the embryonal frog in coagulated frog lymph, showed that from the neuroblasts of His hyaline threads grew out which increased in length by means of ameboid movements. Harrison has interpreted these threads as axis cylinders. Burrows observed the same phenomenon in cultures in chick plasma of the medullary cord of a chick embryo two days old, and succeeded in staining the newly formed axis cylinder.[2] Lewis and Lewis[3] have described the growth of sympathetic nerves from the intestines of chick embryos in saline solutions.[4]

I began the experiments, the preliminary results of which are given in this publication, with the cultivation in coagulated chick plasma of the neural tube of chick embryos two to three days old, and in a few cases (5 out of 102 experiments) I observed that after ten to twenty-four hours there grew out from the transplanted piece slender filaments with a bulbular end which, by ameboid movements through the plasma, increased the length of the thread. Besides, there occurred in all the cultures a rich proliferation of the

* Received for publication, November 2, 1912.

[1] Harrison, R. G., *Jour. Exper. Zoöl.*, 1910, ix, 787; *Anat. Rec.*, 1912, vi, 181.

[2] Burrows, M. T., *Jour. Exper. Zoöl.*, 1911, x, 63.

[3] Lewis, W. H., and Lewis, M. R., *Anat. Rec.*, 1912, vi, 7, 207.

[4] Since writing this paper, an article by Marinesco and Minea (Essai de culture des ganglions spinaux de mammifères *in vitro*, *Anat. Anz.*, 1912, xlii, 161) has come to my attention. These authors have cultivated spinal ganglia of young cats and rabbits in plasma in large dishes. They observed growth of connective tissue cells and of axis cylinders, which were stained according to the method of Cajal. The newly formed nerve fibers are described in some cases as prolongations of the cell body ending in swellings, in other cases as pericellular networks and plexuses.

embryonal connective tissue cells, and this was probably the cause of the scant percentage of successful cultures, for we know that the growth of connective tissue prevents the development of tissues of a higher order.

I began, therefore, to cultivate the tissues of the central nervous system of chick embryos in a more advanced and differentiated stage and found that pieces of the cerebral cortex of chick embryos six to seven days old gave 100 per cent. of successful cultures as far as the growth of the axis cylinders is concerned.

The cultures were prepared in the ordinary way as described for cultivation of tissues in general by Carrel and Burrows. The embryo was taken out of the egg and placed in Ringer solution heated to 40° C. From the forebrain the covering membranes were removed by means of scissors and forceps, and then, with a cataract knife, very thin slices of the cortex, about one millimeter square, were taken out. These slices were put immediately at room temperature in a drop of plasma on a cover-glass, and the cover-glass was inverted over a hollow slide, without waiting for the coagulation of the plasma. In some cases the plasma was diluted one third or one fourth with distilled water. In other cases it was not diluted. In this way it was possible to prepare each culture in one half or one quarter of a minute. In some cases the tissues were placed on a piece of silk veil in the plasma, the silk being a support for the growing filaments. I shall now describe the phenomena of growth that occur in a culture of the brain of the chick embryo six to seven days old.

After incubating the cultures for ten, eighteen, and twenty-four hours, a number of thin, slender, light-breaking filaments consisting of hyaline protoplasm without granules or visible structure were seen growing out from the pieces. Most of these filaments were of the same thickness, about one half to one micron. Throughout their length they were curved and bent in several directions. After twenty-four hours, most of them reached a length of 250 to 300 microns, and a few even 460 microns. Several of these filaments ended in small swellings of the thread (figure 1). This bulb was sometimes regular and ovoid, and measured two to three microns in width. Some of the filaments ended in a point, and a few had

finger-like branches in which ameboid movements could be observed. These filaments might be present in small numbers in each culture, or they might grow so densely that they formed a real tissue around the fragment of brain. A few of them had branched by the twenty-fourth hour.

After forty-eight hours the fibers increased in length and thickness. Some of the fibers now reached a length of about one millimeter, and one of them was 1.1 millimeters. Some of the thickest fibers showed a plain fibrillation in their central parts, and further in their course swellings were seen. These swellings were of different size and shape. Most of them were slight and somewhat irregular, but in some cases they resembled very much the end bulb of the filament. They consisted always of hyaline protoplasm. Branching of the thickest fibers was frequently observed at this stage, and anastomoses between different branches occurred in some places (figure 2). After forty-eight hours another feature of interest was observed, that is, the occurrence of isolated cells lying free in the plasma near the border of the fragment of tissue. These cells were of two kinds. (1) Large cells, without any definite cell membrane, with many granules, and one large, clear nucleus. They sometimes have one or two short protoplasmatic, filamentary processes, and in a few cases I observed one or two of the above mentioned long protoplasmatic threads with bulbular ends growing out from these cells (figures 3 and 4). (2) Cells of another type which were mostly oblong, sometimes fusiform with sharp outlines. These cells had a very definite cell membrane, one nucleus, and powerful, straight, tapering outgrowths, three to five microns wide, in which granules were visible. The outgrowths never had bulbular endings and were easily distinguished from the above mentioned slender curved hyaline filaments (figure 5). There is no reason to doubt that the cells of this second type are connective tissue cells (glia (?), endothelial (?)), while the first type is similar in character to ganglion cells, and its outgrowths are very much like the filament described by Harrison in the cultures of embryonal frog, and in all probability are axis cylinders.

On the third day there was still some increase in size of these nerve fibers and the longest ones observed were 1.25 millimeters in length.

On the fourth day but little difference was seen. Granulation occurred in some of the fibers and their outlines became less definite. On the fifth and sixth days the fibers disappeared completely.

I continued to work with the brains of chick embryos of different ages. As far as the growth of nerve fibers was concerned a high percentage of the cultures was successful. A few cultures were discarded as not useful on account of technical errors.

TABLE I.

Cultures of Brain of Chick Embryo of Different Ages.

Age of the embryos.	No. of animals employed.	Total No. of cultures.	No. of cultures in which axis cylinders were present.	Percentage of growth of axis cylinders.
2 days	4	19	2	10.5
3 days	10	83	3	3.6
6–10 days	5	32	32	100.0
11–15 days	6	65	49	75.3
16–21 days	8	53	37	69.8
		252	123	

The growth of the axis cylinder from brains of chick embryo of a more advanced age showed practically the same characteristics as described formerly for the six to seven day old embryos. There were no striking differences in the rate of growth. In one of the cultures, there occurred a phenomenon which deserved closer attention. The culture was from the brain of a chick embryo eighteen days old (figure 6). On the third day there had developed a great number of thick and thin axis cylinders which branched and anastomosed, forming a network. The swellings of these fibers were larger than any observed earlier. They appeared like refractive homogeneous globes, four to five microns in diameter, lying in the course of the filament or at the anastomosis between two or three filaments. Sometimes they were oblong, and in shape were very much like bipolar ganglia cells. As they had no nucleus, however, I am sure they were not. I consider them as accumulations of protoplasm analogous to that in the end bulb, and like the latter they give rise to outgrowths of new fibers.

Having established these facts I tried in some few cases to cultivate the spinal cord of chick embryos and succeeded in observing

axis cylinders sprouting out from the pieces of tissue (figure 7). Next I began the cultivation of the cerebral nervous systems of young mammals. Rabbits, cats, and dogs were employed, and preparations were made from the brain, the cerebellum, the spinal cord, and the spinal ganglia. The animals were operated upon under ether anesthesia. The tissue was put directly from the animal into Ringer solution at 40° C., and was then cut into small pieces. The preparation of each culture took about half a minute. A little more time was required for the dissection and cutting of the spinal ganglia with their tough connective tissue envelopes.

TABLE II.

Cultures of the Central Nervous System of a Rabbit Two Months Old.

No. of animals employed.	Origin of cultures.	Total No. of cultures.	No. of cultures in which axis cylinders were present.
2	Brain	8	2
	Spinal cord	14	1
	Spinal ganglia	5	0

TABLE III.

Cultures of the Central Nervous System of a Rabbit Seven Months Old.

No. of animals employed.	Origin of cultures.	Total No. of cultures.	No. of cultures in which axis cylinders were present.
3	Brain	23	0
	Spinal cord	4	0
	Spinal ganglia	4	2

TABLE IV.

Cultures of the Central Nervous System of a Cat Six Weeks Old.

No. of animals employed.	Origin of cultures.	Total No. of cultures.	No. of cultures in which axis cylinders were present.
3	Brain	24	3
	Spinal cord	7	1
	Spinal ganglia	12	2

The development of the axis cylinder in cultures from these young animals was slower than in those from embryonic nervous tissue. They were observed usually after thirty-six or forty-eight hours, and only a few were present in each culture. They showed,

however, the same main characteristics as those from embryonic chicks. The filaments were curved, hyaline, ended usually in a bulb, had branches, and were longitudinally striated when they had reached a certain size. In some of them, especially in those prepared from the gray substance of the spinal cord and from the spinal ganglia, granulations were visible (figures 8 and 9).

The cultures prepared from the brain of these animals showed a development of connective tissue cells only after three days, and, fortunately for the axis cylinders, only a few connective tissue cells appeared in each of the cultures, while the spinal ganglia gave rise to a rich growth of connective tissue cells.

The cultures of the nervous system from rabbits were kept for observation for only three days and a half, were then recorded as positive or negative, and the negative ones were discarded. It is possible, as was learned later, that the results obtained might have been changed and might have shown a greater number of positive cultures if they had been kept for observation for five or six days. In adult animals it is necessary to observe the cultures for a long time to be sure that they will not develop axis cylinders. If they do develop, they live for only a very short time. I have never seen axis cylinders which did not undergo degeneration after the seventh day of the incubation of the culture at 40° C.

I had, however, not yet reached my goal, which was to observe the development of axis cylinders in cultures of the cerebral nervous system of mammals in such a large percentage of the cases that it might be useful for the study of degeneration and regeneration. I succeeded when I began to work with dogs. From the brain of a dog three weeks old I observed a growth of axis cylinders. This growth took place from the cerebrum, but still more abundantly from the cerebellum. Of fifteen cultures prepared from the brain of a dog three weeks old, eleven were positive, and most of these showed an abundance of axis cylinders (figure 10). Out of ten cultures prepared from the cerebellum of two such dogs, every one showed a great number of axis cylinders; that is, 100 per cent. of the cultures were successful.

After twenty-four hours the axis cylinders from the brain of this dog had already appeared, and those from the cerebrum showed

the characteristics mentioned above for the chick, cat, and rabbit. The growth from the cerebellum I will mention a little more particularly (figure 11). These nerve fibers have usually a big bulb at their ends and are bent in curves and angles. They are richly provided with swellings and form branches and anastomoses within the first twenty-four hours. After two or three days the fibers were rather thick (from two to three microns), and some of them showed a faint fibrillation. The swellings now have become very numerous and have a diameter of four to five microns; they are either globular or oblong and are quite homogeneous. After five days' incubation the length of these fibers was 0.9 of a millimeter.

On the fifth day these fibers showed the following degenerative changes: A faint granulation appeared first in the swellings and shortly afterward in the fibers. The outlines of the fibers became less definite, the fibers and the swellings became less refractive, and the edges of the globular swellings appeared as if they had been nibbled. The change was seen at the same time throughout the whole length of the fiber and increased during the next two or three days, so that on the seventh day after incubation all the fibers appeared degenerated.

EXPERIMENTAL PART.

I shall describe in detail one of the experiments in which I cut some of the nerve fibers in the cultures of the cerebellum of dogs three weeks old.

After twenty-four hours a large number of axis cylinders had developed, which increased in size during the following two days. On the fourth day I cut through the plasma and some of the nerve fibers contained in it close to the piece of tissue (figure 12). As the coagulated plasmatic jelly is under tension the cut in it produced a small gap by the retraction of the medium. This was immediately filled with fresh plasma from the same animal, which had been kept on ice, and which now coagulated. The cover-slip was again inverted on a hollow slide and incubated. It was observed from hour to hour until late in the evening. The cutting of the plasma had disturbed slightly the central part of the cut nerve fibers, as the retraction had taken place especially at this side. It was observed immedi-

ately after section that the central part of the nerve fibers had been a little crumpled and their outlines were more curved than before (figure 12). This was not true of the peripheral part in which no visible changes took place either at the time of the cutting or in the following nine hours. The next morning, however, that is, twenty hours after the cutting, changes of a different kind had occurred, degenerative as well as regenerative (figure 13). The cut fibers were thinner than on the previous day. This could be determined by comparing them with nearby fibers which were not cut, and which before section were of the same thickness. Moreover, the cut fibers had a faint granulation and the edge of the swellings appeared moth-eaten. The appearance of the central part of the cut fibers had not changed visibly. But in the gap that was filled with plasma there had grown out from the central part of the cut fibers three axis cylinders, of which the longest one, grown during the lapse of twenty hours since the cutting, had a length of 150 microns. There was nothing else extraordinary about them. They looked like the other young axis cylinders, had end bulbs and some swellings. Their growth was followed during the next two days, during which time one of them increased sixty-five microns in length, the others not quite so much (figure 14).

Meanwhile the degeneration in the peripheral part of the cut fibers continued slowly throughout its entire length (figures 14 and 15). The fibers became thinner and less refractive, and the granulations increased. At last, on the sixth day, they could hardly be seen. At this time the other fibers which had not been cut were partly granular but their outlines were still distinct (figure 15). The specimens were stained after three and six days.

The technique which after several attempts was found to give the most successful preparation was the following:

1. Fixation in 2 per cent. formalin for 15 to 18 hours.
2. Staining in a very dilute solution of Held's molybdic acid hematoxylin (6 to 8 drops to 15 c.c. of water) for 12 hours.
3. Differentiation in a dilute solution of the Weigert borax-potassium-ferrocyanide solution (2 drops to 20 c.c. of water) for 12 to 20 hours.
4. Dehydration, xylol, Canada balsam.

The color of the fibers is greyish blue, and they are very distinct. The method seems to injure the fibers a little, for even when fixed

and stained after three days' incubation the stained specimens are plainly granular, while the living fibers show no granulation at all. I have, therefore, studied mainly the living specimens, and most of the illustrations given here are camera lucida drawings of living fibers. The camera lucida was employed because it is hardly possible to get a photograph of one nerve fiber in its whole length, as it curves and bends through many planes, and living fibers were drawn because these are of course of greater interest than those that are dead.

CONCLUSIONS.

1. The brains of chick embryos, of cats six weeks old, of rabbits two months old, and of dogs three weeks old, when cultivated *in vitro,* develop long filaments which, according to their growth and their anatomical and tinctorial characters, must be considered as true axis cylinders.

2. Similar structures develop from spinal ganglia of rabbits seven months old, and from the spinal cord of cats six weeks old, and of rabbits two months old.

3. When severed from their origin by section these threads undergo degenerative changes which do not appear after nine hours, but which are seen after twenty hours, and continue until in the course of the following two days the thread degenerates completely.

4. After twenty hours the development of new axis cylinders from the central part of the cut fibers is observed.

EXPLANATION OF PLATES.

PLATE 28.

FIG. 1. Culture of brain cortex twenty-four hours old from a chick embryo six days old. Culture on silk veil.

PLATE 29.

FIG. 2. Culture of brain cortex two days old from a chick embryo fourteen days old.

PLATE 30.

FIG. 3. Culture of brain cortex forty-eight hours old from a chick embryo six days old. Isolated cells, one of them with an axis cylinder.

PLATE 31.

FIG. 4. Culture of brain cortex forty-eight hours old from a chick embryo twelve days old. Isolated ganglion cell with axis cylinder.

FIG. 1.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

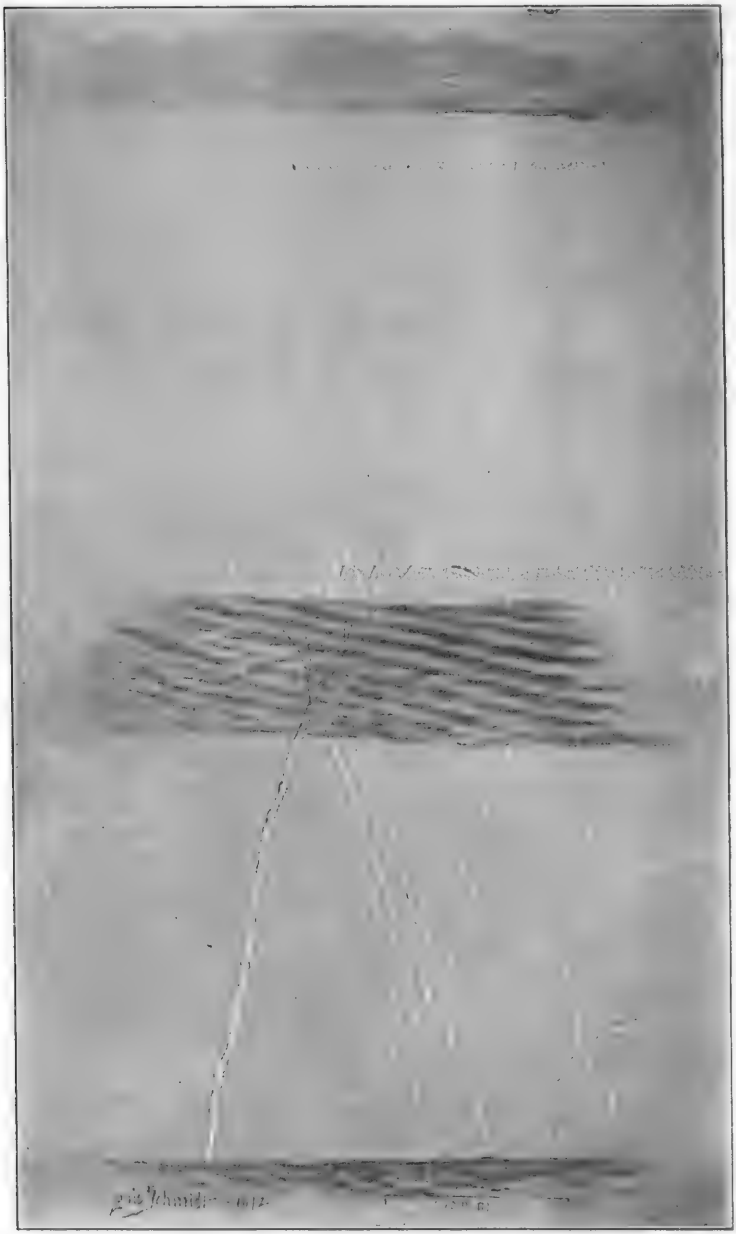

FIG. 2.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

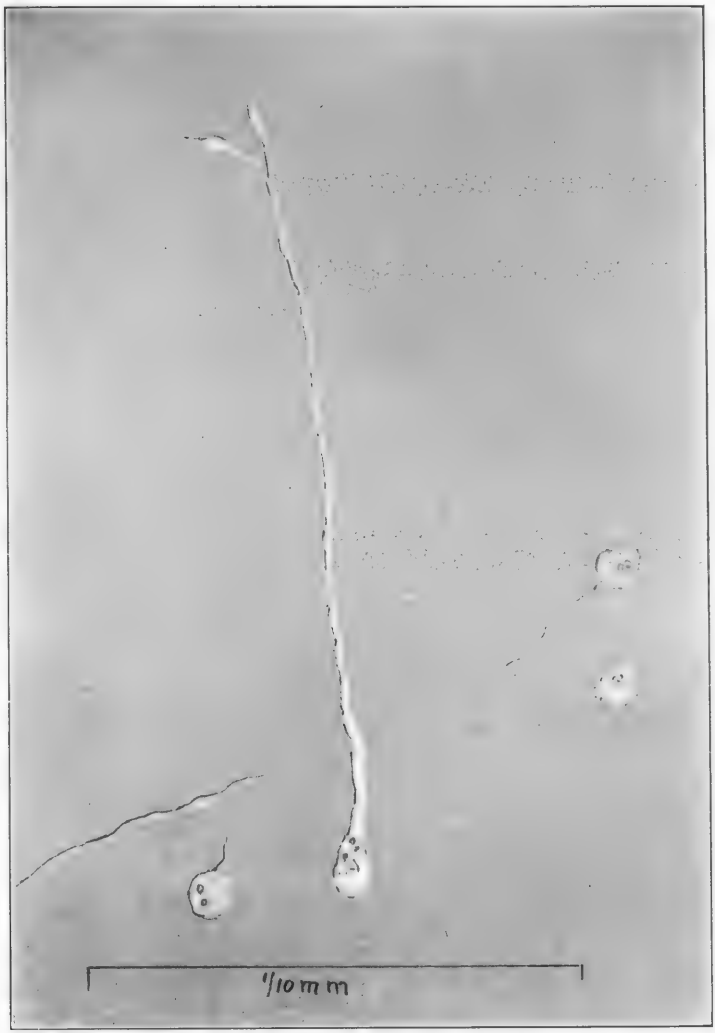

FIG. 3.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 4.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 5.

Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 6.

FIG. 7.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 8.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

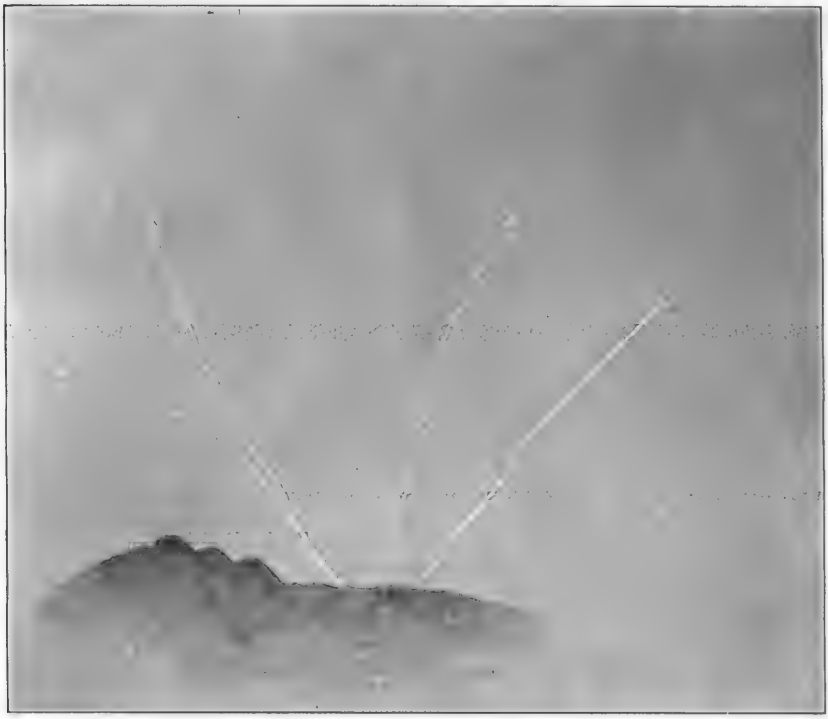

FIG. 9.

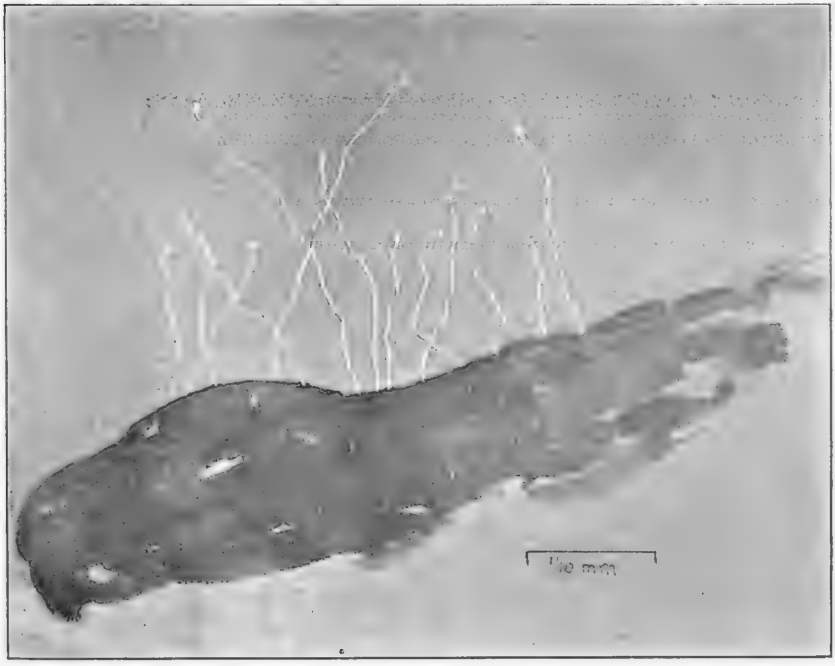

FIG. 10.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 11.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 12.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 13.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

FIG. 14.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

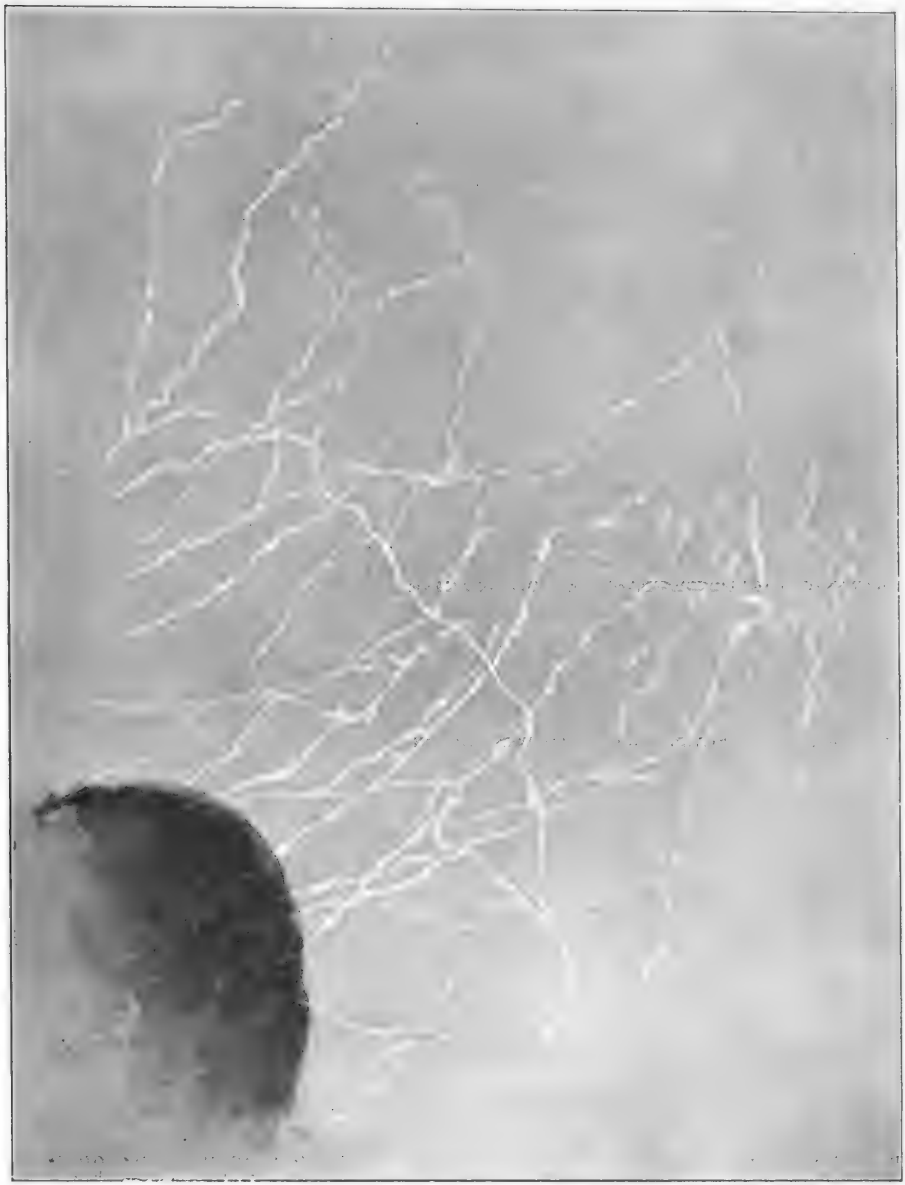

FIG. 15.

(Ingebrigtsen: Degeneration and Regeneration of Axis Cylinders *in Vitro*.)

PLATE 32.

FIG. 5. Connective tissue cells from a four day old culture of spinal ganglion of a cat six weeks old. Stained with hematoxylin.

PLATE 33.

FIG. 6. Culture of brain cortex three days old from a chick embryo eighteen days old.

FIG. 7. Culture of spinal medulla three days old from a chick embryo fourteen days old.

PLATE 34.

FIG. 8. Culture of spinal ganglion four days old from a rabbit seven months old.

PLATE 35.

FIG. 9. Culture of spinal ganglion two days old from a rabbit seven months old.

FIG. 10. Culture of brain cortex twenty-four hours old from a dog three weeks old.

PLATE 36.

FIG. 11. Culture of cerebellar cortex three days old from a dog three weeks old. Figures 12 to 15 are made from this culture in different stages.

PLATE 37.

FIG. 12. Culture of cerebellar cortex three days old from a dog three weeks old immediately after the cutting of the fibres. The gap is visible.

PLATE 38.

FIG. 13. Culture of cerebellar cortex four days old twenty hours after the cutting of the fibers which are slightly degenerated. New axis cylinders are seen in the gap.

PLATE 39.

FIG. 14. Culture of cerebellar cortex five days old forty-four hours after the cutting of the fibers which are still more degenerated. The new fibers in the gap have grown a little longer; one of them is slightly degenerated.

PLATE 40.

FIG. 15. Culture of cerebellar cortex five days old from a place just beyond the cut fibers. These axis cylinders are not degenerated.

INTESTINAL OBSTRUCTION IN THE RABBIT. I.*

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Although an extensive literature has grown up concerning the cause of death in high intestinal obstruction, there appears to be as yet no unanimity of opinion on the subject. Even in the articles which have been published during the past year there is almost as wide disagreement as in the older literature. It seems desirable, therefore, to add the weight of the results of the experimental study here reported to that side of the controversy on which they lie.

In view of the recent summaries by Murphy and Vincent,[1] and by Hartwell and Hoguet,[2] it seems unnecessary to review the older literature on the subject. It may suffice to say that of the older theories as to the cause of death in intestinal obstruction but one has survived which has any standing among present investigators. The theory that the fatal effect of obstruction is due to a nervous reflex has been given up because of lack of evidence to support it, while the theory that death follows a general bacterial invasion of the peritoneum or of the blood stream has failed in the face of strong evidence against it. The surviving older theory is that death is due to the absorption of a toxin or of toxins from the lumen of the obstructed gut. That there is a toxic factor in high intestinal obstruction is accepted by the majority of those who have recently published on the subject, but it is accepted with various reservations and with points of difference as to the source of the toxin.

Maury[3] (Draper)[4] furnishes evidence in favor of the theory that the toxic substance is secreted by the duodenal mucosa, and that this substance in health

* Received for publication, November 9, 1912.

[1] Murphy, F. T., and Vincent, B., *Boston Med. and Surg. Jour.*, 1911, clxv, 684.

[2] Hartwell, J. A., and Hoguet, J. P., *Am. Jour. Med. Sc.*, 1912, cxliii, 357.

[3] Maury, J. W. D., *Am. Jour. Med. Sc.*, 1909, cxxxvii, 725.

[4] Draper, J. W., *Jour. Am. Med. Assn.*, 1911, lvii, 1338.

is neutralized by secretions from the mucosa of lower segments of the intestine. In cases of obstruction he found that the animal's life may be prolonged by feeding it jejunal mucosa.

Stone, Bernheim, and Whipple[5] have demonstrated in the obstructed duodenal loop in dogs a toxic substance which is not injured by heating at 60° C. for long periods, which can not be filtered out of the fluid, can not be destroyed by autolysis, by pancreatic digestion, or by bacterial fermentation, and which they believe to be a secretion from the duodenal mucosa, though they were unable to obtain a similar toxic substance by any chemical action upon the normal mucosa.

Murphy and Vincent[6] conclude that the vital factor in the production of the symptoms of acute ileus is the interference with the circulation of the obstructed intestinal segment, particularly the interference with the venous outflow, while the acute symptoms are caused by the absorption of a toxic substance which they believe is purely of bacterial origin. They consider that death is due to the living bacteria with their endotoxins.

Hartwell and Hoguet[7] in their first paper emphasized injury to the intestinal wall as the vital factor in causing death, by permitting the absorption into the blood stream of such toxic substances, of whatever origin, as are normally present in the intestinal lumen, or as are formed after obstruction. In a more recent paper,[8] as the result of further experimentation, they have advanced a new theory, to the effect that "the important element in the development of the symptoms seen in intestinal obstruction in dogs is the loss of water due to the vomiting," and have given evidence to show that life in animals with obstruction may be prolonged by subcutaneous administration of salt solution.

The authors quoted, with the exception of Hartwell and Hoguet, agree that high intestinal obstruction is more rapidly fatal than low obstruction. Hartwell and Hoguet could find no difference, but maintain that double ligation with isolation of a loop is more rapidly fatal than a single ligation, as the injury to the mucosa is greater in the former case.

The series of experiments forming the basis of this paper was the outgrowth of class experiments on the effect of intestinal obstruction, and differs from the experiments reported by the majority of observers in that the animal used for study was the rabbit and not the dog. The rabbit is, from one standpoint, an unfavorable animal for these experiments, as it is impossible with any reasonable procedure to secure the animal with an empty stomach. On the other hand, it is a particularly satisfactory animal for the study of duodenal obstruction because of the long loop of duodenum

[5] Stone, H. B., Bernheim, B. M., and Whipple, G. H., *Bull. Johns Hopkins Hosp.*, 1912, xxiii, 160.

[6] Murphy, F. T., and Vincent, B., *loc. cit.*

[7] Hartwell, J. A., and Hoguet, J. P., *Am. Jour. Med. Sc., loc. cit.*

[8] Hartwell, J. A., and Hoguet, J. P., *Jour. Am. Med. Assn.*, 1912, lix, 82.

(twenty-five to thirty-five centimeters) lying between the entrance of the bile duct and that of the pancreatic duct. This gives opportunity for cutting out these secretions and thus for the study of the unmixed secretion from the isolated duodenal mucosa.

In conducting the experiments the operative procedure was carried out under ether anesthesia, which, as a rule, had a total duration of about twenty minutes. Uniform and careful surgical technique was employed and we were successful in preventing any complication from infections. The agent used to produce obstruction was a coarse, soft, twine ligature, carefully applied so as to produce complete obstruction with the least injury to the intestinal wall or to the blood vessels. In order to secure this latter point, the ligature was passed between the intestine and the vascular loops lying in the mesentery close to the intestinal wall. To the majority of animals operated upon no food was given for twenty-four to forty-eight hours previous to operation, and water only was given subsequent to the operation, as it was found early in the work that continued feeding led to an earlier lethal result, and in some cases to such accidents as perforation of the stomach.

The preliminary set of experiments showed that in the rabbit, as in the dog, there was a striking difference in the time ensuing between operation and death, in animals in which the obstruction was produced at different levels. Duodenal obstruction with the ligature twenty-five centimeters from the pylorus produced death in less than twenty hours, while after obstruction in the upper ileum, 100 centimeters from the pylorus, the animal survived practically forty hours, and with the ligature placed below the cæcum, the animal lived eleven days. The short interval between obstruction and death in the animal with duodenal ligation seemed to indicate that bacteria played no part in the fatal result, at least in an infectious way, but that death was due to a toxin of some nature.

As the work of Bunting and Brown,[9] in this laboratory, had shown that rabbit bile was extremely toxic when reabsorbed from the peritoneal cavity, it seemed necessary to rule out this secretion as a factor, especially as at the time these experiments were begun the published articles had not settled the point. The rabbit stands

[9] Bunting, C. H., and Brown, W. H., *Jour. Exper. Med.*, 1911, xiv, 445.

well the ligation of the bile duct, surviving a month if there be no leakage of bile into the surrounding tissues. It was a simple matter, therefore, to rule out the bile as a factor in the process by ligation of the duct previous to the obstructing operation. In rabbit 5 of the series the duct was ligated seven days previous to the duodenal obstruction, and death followed the latter in about twenty-two hours. In rabbits 21, 22, and 25, ligation of the bile duct was done on the day previous to the obstruction and the fatal result followed in each case in less than twenty-four hours. In many of the subsequent experiments the bile duct and pylorus were occluded by a single coarse ligature in order to secure the duodenal secretions freed from gastric contents and bile, and in no case was the course of the subsequent duodenal obstruction modified.

That the pancreatic secretion plays no part was shown easily in the rabbit, on account of the position of the duct. In practically the entire series of duodenal obstructions the ligature was placed above the duct, thus causing no confinement of that secretion. Experiments had also shown that a rabbit in which the pancreatic juice pours directly into the peritoneal cavity through a severed duct may survive for a week.

The effect of ligation of the pylorus was next studied and it was found that, in the rabbit, obstruction at that point will cause death constantly in from thirty-six to forty-eight hours. The reabsorption of gastric secretion and the products of digestion have a sharp toxic effect (rabbits 17, 19, and others). Death from pyloric obstruction is never so prompt, however, as that following duodenal obstruction, or as that following the combined pyloric and duodenal obstruction.

The experimental results which had been secured up to this point indicated that in the rabbit there was a very striking and interesting relation between the position of the intestinal ligature and the interval elapsing between operation and death. The series was extended further and this point came out even more sharply. Indeed it may be said that in a series of about forty animals operated upon, the results as summarized below have been constant; that is, in animals weighing about 2,000 grams and deprived of food for from thirty-six to forty-eight hours previous to operation. It seems to

be unnecessary to record the details of operations, as the technique followed in each case has already been indicated.

The results of the series are as follows: (1) Obstruction of the pylorus in the rabbit is followed by death in from thirty-six to forty-eight hours, unless perforation of the stomach ensues, in which case death will occur sooner (rabbits 17, 19, and 20). (2) Obstruction of the duodenum between the pylorus and a point about eight centimeters below is followed by death in from thirty-six to forty-eight hours (rabbits 31, 32, 53, 56, and 58). (3) Obstruction at a point from eight to ten centimeters below the pylorus will cause death in approximately twenty-four hours (rabbits 4, 5, 52, and 61). (4) Obstruction of the duodenum below this point will be followed by death in less than twenty-four hours, often in less than fifteen (rabbits A, 1, 2, 21, and others). (5) Obstruction of the jejunum to a point approximately ninety centimeters below the pylorus will result in death in about twenty-four hours (rabbits 27 and 65). (6) Obstruction of the ileum may cause death in from thirty-six to forty-eight hours, although when the ligature is placed close to the cæcum life may be prolonged for a week (rabbits 28, 30, 66, 67, 74, and 75). (7) With obstruction below the cæcum, the animal may survive still longer (rabbit C, eleven days).

These relations seem to be constant unless modified by continued feeding, when the interval is shortened, especially in high obstructions, or by some physiological or pathological process, such as an existing pregnancy or an infection. No difference in time reaction has been noted between the use of a double ligature isolating a loop, and that of a single ligature.

In studying the series of experiments it appears that obstruction of the duodenum holds a peculiar position by reason of the great rapidity of the onset of symptoms and the short interval before death. The animal appears to be overwhelmed by a toxin. Lethargy, muscular twitchings, and a rapid fall of temperature to a point in the neighborhood of 90° F., or even lower, are seen just before death. Pulse rate and respiratory rate are both decreased toward the termination. It has been suggested that in cases of obstruction these symptoms might be due to the lack of an internal duodenal secretion. This can be disproved not only by excision of

the duodenum, but by the positive results obtained with injections of the duodenal contents. In simple excision of the duodenum, with ligation and cauterization of the pyloric and jejunal stumps, and without gastro-enterostomy (a somewhat difficult procedure in the rabbit), the animal will live for the same length of time as with a simple pyloric obstruction, that is, about forty to forty-eight hours, and the course of symptoms is in no way different from that in simple obstruction. Injection experiments have shown that the fluid in a closed duodenal loop is toxic, and while a report of the experiments concerning the nature of the secretion and its pathological effects is reserved for a subsequent paper, it may be stated that a closed duodenal loop will be distended within three hours after placing the ligature, by a fluid which is clear, slightly straw colored, of about the same alkalinity as the pancreatic juice, and containing considerable mucin. Intravenous testing of the fluid is impractical because instant death follows the injection of small amounts. The autopsy, made immediately after death, shows soft agglutinative clots in the right heart. The intraperitoneal injection of about twenty cubic centimeters kills a rabbit weighing 1,000 grams in less than twenty hours, causing before death the rapid fall of temperature, the muscular twitching, and the slowing of the heart seen in the obstruction cases.

This toxin seems to be a secretion from the duodenal mucosa. The poverty of this portion of the intestine in bacteria, combined with the appearance of toxicity in the fluid so shortly after ligation, seems to weigh against the bacteria present having anything to do with the process. It seems also improbable that constant symptoms as seen in obstruction would follow the action of the variegated flora found in the animal's intestine.

Assuming then that a toxic secretion is present,—and every evidence from our series is in favor of this point of view,—it seems apparent from the time relations given above, that a certain length (about ten centimeters) of duodenum must lie above the ligature before the toxic dose secreted rises to the point necessary to kill in twenty-four hours or less. And further, that with the ligature below the duodenum, a certain length of intestine (about 100 centimeters), as suggested by Draper (Maury),[10] must be allowed to act

[10] Draper, J. W., (Maury), *loc. cit.*

upon this toxic secretion before its tendency toward a rapid fatal effect can be overcome.

Withdrawal of fluid from the body by vomiting can not be a factor in the rabbit. Vomiting does not follow obstruction in the rabbit except as an agonal occurrence. As was stated above there is a rapid secretion of fluid into the intestine in rabbits with obstruction, but in low obstruction, with all loops of intestine distended to the utmost, there is obviously much more fluid withdrawn from the tissues than in obstruction of a ten centimeter length of duodenum, and in the former case life may last a week, in the latter less than a day.

One should be able to save the life of an animal with obstruction by duodenostomy, and although Stone, Bernheim, and Whipple[11] have succeeded in the dog, our efforts with the rabbit have been successful only in prolonging life in duodenal obstruction by duodenostomy to forty-eight hours instead of the usual twenty hours or less. The result is probably due to a faulty technique, perhaps to absorption of secretion through the wound edges, as a similar duodenostomy without obstructing ligature gave the same fatal effect in forty-eight hours.

The import of this series of experiments is to the effect that the reabsorption of a toxic secretion from the lumen of the duodenum is the important factor in causing the rapid death of rabbits after high intestinal obstruction.

[11] Stone, H. B., Bernheim, B. M., and Whipple, G. H., *loc. cit.*

INTESTINAL ABSORPTION IN INFANTILISM.*

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Previous investigation has shown that in certain cases of infantilism the amount of nitrogen and other elements in the urine is very low, and the amount of these elements in the feces is high. It occurred to us that the losses through the feces might be responsible for the low plane of metabolism and for the failure to develop. To test this possibility, a diet rich in protein was fed for a long period to a dwarf (F. S., reported in earlier papers). Losses of nitrogen through the feces due to inadequacy in the digestion or absorption of protein would, of course, be exaggerated by the high protein diet and would give rise to even higher values for the nitrogen of the feces. At the same time such a diet should raise the plane of metabolism and lead to improvement in the clinical condition.

A high protein diet was maintained for over two months, but attacks of eczema, possibly attributable to the diet, led to its discontinuance, so that not much stress can be laid on the clinical observation that there was no improvement in growth. The chemical examinations were more helpful. Table I shows the results of examinations made before the high protein diet was begun. and table II those made after the diet had been continued for several weeks.

TABLE I.

Date.	Urine.						Nitrogen in feces.
	Total nitrogen.	Kreatinin.	Kreatin.	Calcium oxide.	Magnesium oxide.	Phosphorus pentoxide.	
Oct. 23, 1911	4.54	0.253	0.107	0.0144	0.223	1.230	1.34
Oct. 24, 1911	2.70	0.238	0.022	0.0192	0.133	0.772	3.32
Oct. 25, 1911	2.66	0.205	0.048	0.0112	0.140	0.780	1.75
Oct. 26, 1911	2.50	0.213	0.034	0.0110	0.130	0.832	1.48

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TABLE II.

Date.	Urine.						Nitrogen in feces.
	Total nitrogen.	Kreatinin.	Kreatin.	Calcium oxide.	Magnesium oxide.	Phosphorus pentoxide.	
Dec. 14, 1911	12.50	0.386	0.800	0.0156	0.1254	1.633	1.076
Dec. 15, 1911	15.36	0.425	1.015	0.0224	0.1688	1.991	1.225
Dec. 16, 1911	20.80	0.519	1.864	0.0340	0.1830	2.581	1.931
Dec. 17, 1911	12.88	0.337	0.863	0.0216	0.1712	1.822	0.797

In spite of the fivefold increase of food protein in the second period, the nitrogen of the feces is not only not increased, but actually decreased,—a finding indicating that the high nitrogen of the feces on the usual diet can not be due to any inadequacy in the digestion or absorption of protein. The results are in accord with those of previous experiments[1] in indicating that the nitrogen in the feces in infantilism is of the same origin as that in normal feces, being chiefly excretory and not representing unabsorbed food residue.

The increased excretion of nitrogen, phosphate, and, to a less extent, magnesium in the urine on a high protein diet is further evidence that there is no considerable disturbance in the digestion or absorption of protein. It is evident too that we can raise the plane of the metabolism of the elements of the soft tissues without affecting either growth or the metabolism of calcium.

The effect of the high meat diet on the kreatin excretion is important. It will be observed that the amount of kreatin excreted runs parallel day by day with the total nitrogen excretion, so that at least some of the kreatin excreted must have an exogenous origin. Folin[2] has found that kreatin of the food appears again in the urine only when the nutrition of the individual is on a high plane, so that it is fair to assume that the nutrition of our patients is on a high plane. The results seem to exclude the possibility that the failure to develop depends on a condition of malnutrition of the soft tissues or on a fundamental inability to engage in a higher rate of metabolism.

[1] McCrudden, F. H., and Fales, H. L., *Jour. Exper. Med.*, 1913, xvii, 20.

[2] Folin, O., The Chemistry and Biochemistry of Kreatin and Kreatinin, Olaf Hammarsten, Festschrift, Upsala, 1906.

The results are in accord with those of our previous work in indicating that there is no disturbance in the digestion, absorption, or utilization of protein in the dwarfs of this type. Large quantities of protein food are digested and absorbed as readily as small quantities without any observable favorable effect on the skeletal abnormalities or on the abnormalities in the calcium metabolism. The general nutrition appears to be good and on a high plane.

THE CAUSE OF THE FAILURE TO DEVELOP IN INFANTILISM.*

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Changes in metabolism have been found in certain forms of dwarfism.[1] The nitrogen, magnesium, and phosphate of the urine were low, and the calcium of the urine was so low as to be practically negligible. The feces, on the other hand, were large and contained in great excess the various elements which were so low in the urine, the amount of calcium being even greater than that in the food. In other words, the metabolism was found to be on a very low plane, and there was considerable loss of material, especially calcium, through the feces.

One of our patients, F. S., was under observation and treatment for a long period, and some of the clinical features of the disorder showed marked improvement.

In November, 1910, the patient was a puny little child, so weak that he lifted his feet with difficulty in walking. His height was 96 cm., and his weight 13.6 kilos. He had very poor muscular control and when lying on his back was unable to lift his trunk to the sitting position by contraction of the abdominal muscles. The stools were large and in odor very offensive, and only the smallest quantities of food could be given without causing increased foulness.

By May, 1911, there was a striking change. He had become a fat, rosy, cheerful, healthy boy, could run, and had excellent muscular control. His weight had increased to 20 kilos, but his growth had been slight, an increase of about 1 cm. in height during the winter.

Briefly stated, there appeared to be a striking improvement in the general health and nutrition of the soft tissues, but the abnormalities referable to the skeletal system did not improve. The bones remained small and fragile and the patient did not grow.

In view of the number of changes in metabolism which had previously been found, it seemed worth while to carry out in June, 1911,

* Received for publication, November 8, 1912.

[1] McCrudden, F. H., and Fales, H. L., *Jour. Exper. Med.*, 1912, xv, 450.

at a period when the general health had so much improved, a complete balance metabolism experiment similar to that carried out in December, 1910, with the hope of finding which of the metabolic disturbances were fundamental and associated with the failure of the skeletal system to develop.

Table II shows the results of the observations. The analytical and other technical details of the observations have already been described. The results of the previous balance experiment are given in table I for comparison.

TABLE I.

Day.		Total weight of dried feces.	Nitrogen.	Calcium oxide.	Magnesium oxide.	Phosphorus pentoxide
Urine	1		2.75	0.010	0.005	0.512
	2		2.76	0.016	0.070	0.520
	3		3.02	0.021	0.086	0.572
	4		2.75	0.012	0.074	0.497
	5		2.51	0.009	0.054	0.398
	6		2.32	0.040	0.111	0.211
Total.....			16.11	0.108	0.400	2.710
Feces	1	29.60	1.707	1.965	0.213	0.855
	2	14.32	0.868	0.750	0.009	0.767
	3	58.50	3.532	2.744	0.579	2.495
	4	40.35	2.840	1.733	0.339	1.077
	5	52.55	2.286	1.618	0.331	1.072
	6	35.75	2.288	1.451	0.229	1.073
Total.....		231.1	13.52	10.26	1.700	7.34
Total amount excreted .			29.63	10.37	2.10	10.25
Total in food.....			35.64	6.35	2.43	11.91
Retention.....			6.01	——	0.33	1.66
Loss.....			——	4.02	——	——

It will be noted that the quantities of nitrogen, phosphate, and magnesium in the urine have increased considerably, and the phosphate and magnesium of the feces have decreased as the patient has improved. The large stools containing excessive quantities of nitrogen and calcium still persist, and the calcium in the urine remains low.

We have succeeded, then, in improving the general state of nutrition of the patient without, however, improving the disturbance of growth in the skeletal system or correcting the failure to develop. This clinical fact would seem to indicate that the failure to develop

does not depend upon a general nutritional disturbance, but upon some specific disturbance referable to the skeletal system. With the improvement in general nutrition, chemical observations show that the metabolism of those elements associated with the soft tissue has risen to a higher plane. The metabolism in calcium, on the other hand, an element important for bone growth, remains disturbed.

TABLE II.

Day.		Total weight of dried feces.	Nitrogen.	Calcium oxide.	Magnesium oxide.	Phosphorus pentoxide.	Sulphur.
After improvement	1		5.33	0.016	0.210	1.600	0.312
	2		4.26	0.019	0.231	1.268	0.236
	3		5.16	0.026	0.242	1.287	0.317
Urine...	4		5.39	0.022	0.220	1.396	0.348
	5		4.58	0.016	0.218	1.308	0.345
	6		5.84	0.019	0.226	1.495	0.403
	7		5.63	0.018	0.206	1.434	0.452
	8		5.51	0.019	0.257	1.474	0.356
	9		6.04	0.014	0.146	1.285	0.407
	10		7.11	0.029	0.230	1.483	0.467
Total.....			54.85	0.198	2.186	14.03	3.643
Feces...	1	60.91	3.46	1.218	0.237	0.735	0.282
	2	31.83	1.77	0.548	0.096	0.325	0.179
	3	61.91	3.62	1.245	0.225	0.691	0.323
	4	38.60	2.44	0.742	0.109	0.498	0.221
	5	44.77	2.61	0.910	0.107	0.563	0.289
	6	65.40	3.61	1.320	0.256	1.090	0.306
	7	35.97	2.10	0.653	0.100	0.436	0.151
	8	49.32	2.77	0.907	0.170	0.523	0.263
	9	48.53	2.97	0.976	0.192	0.602	0.298
	10	35.88	1.97	0.936	0.165	0.586	0.161
Total.....		473.12	27.32	9.455	1.659	6.049	2.473
Total amount excreted.			82.2	9.65	3.84	20.08	6.12
Total in food.....			110.5	16.85	5.26	31.55	6.55
Retention.....			28.3	7.20	1.42	11.47	0.43

Weight at beginning of experiment, 19.0 kilos; weight at end, 20.2 kilos.

The excessively low calcium of the urine must mean one of three things: (1) either the kidneys are not doing their work properly; (2) the calcium content of the blood is very low; or (3) the calcium of the blood exists in some combination which does not permit of its excretion through the kidneys. There is, however, no other evidence of improper functioning of the kidney, so that one of the

other two conditions must be responsible for the trouble; and in the excessive loss of calcium through the feces, we have a plausible explanation for a low calcium content of the blood.

The results indicate that the general retardation in growth is secondary to a failure to develop on the part of the skeletal system. And the disturbance of calcium metabolism, associated with the frail, thin bones, strongly suggests that the bones have not sufficient calcium in proper form at their disposal. As pointed out by Rubner,[2] there may be two causes for failure to grow: first, a lack of material for growth; and second, an absence of the tendency to grow,—this being the tendency normally seen in the young, but absent in the adult. In the form of underdevelopment described in this paper, there appears to be some disturbance in the supply of material for growth, rather than in the tendency to grow.

[2] Rubner, M., *Arch. f. Hyg.*, 1908, lxvi, 1.

THE RÔLE OF THE LIPOIDS AND PARTICULARLY LECITHIN IN NARCOSIS.*

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The discovery of the anesthetic properties of ether by Jackson and Morton in 1846 led to extensive investigations with the view of finding a common property upon which the narcotic power of the entire group of anesthetics depends. Thirty years later Meyer (1) and Overton (2) formulated their well known theory of narcosis; but it was not long before additional facts presented by various investigators clearly demonstrated the inadequacy of this simple theory and necessitated the advancement of new theories to explain this phenomenon.

It had been known that narcotics are more soluble in fats and oils than in water. With this in mind, von Bibra and Harless,[1] in 1847, carried on a series of experiments on rabbits. Half the animals were anesthetized, and the other half were used as controls. All the animals were killed and the fat content of the brains and livers was determined quantitatively by extraction with alcohol and ether. They found the fat content of the brains of the narcotized animals to be less, and that of their livers greater, than the fat content of these organs in the controls. They, therefore, suggested that narcosis is due to a solution of brain lipoids or fats by the narcotic.

Reicher (3), working in Salkowski's laboratory, repeatedly demonstrated a considerable increase in the total quantity of fat in the blood during and after narcosis, especially with ether or chloroform. He determined the fat content of the blood by extraction with alcohol and ether. Pflüger's school[2] has shown that after digesting the residue obtained by this method with pepsin and again extracting with ether an additional quantity of fat may be obtained. Mansfeld (4) has shown that the total fat obtained from the blood by extracting with ether is only half the actual amount of fat present, and he has further called attention to the fact that the lipemia found in phosphorus poisoning is really due, not to an increase in total blood fat, but to a relative increase in free fat.

* Received for publication, October 5, 1912.

[1] von Bibra and Harless, cited by Meyer, H., *Arch. f. exper Path. u. Pharmakol.*, 1899, xlii, 114.

[2] Cited by Mansfeld, G. (4), *loc. cit.*, p. 47.

Hermann (5) first demonstrated the presence of lecithin in red blood cells and then showed that all narcotics of the aliphatic group were able to dissolve red blood corpuscles and suggested an analogy between the solution of red blood cells, which he attributed mainly to a solution of the lecithins, and the solution of lecithins, cholesterins, and lipoids in the central nervous system during narcosis. Additional strength was given to this hypothesis by the findings of Pohl[3] that, during chloroform narcosis, the greater part of the narcotic in the blood is bound to the red blood cells and particularly to the lecithin and cholesterin constituents thereof; and furthermore, as was to be expected, that the cells of the central nervous system bind still more of the narcotic than the blood, since they are much richer in lecithin and cholesterin. Meyer (1) had previously shown that during alcoholic narcosis the organs that are richer in lipoids contain a much larger quantity of alcohol. Overton (2) maintained that the process consisted in a "solution of the anesthetic in the lipoids, thus changing the normal relationship of these to other cell constituents, but that there was no actual excretion of lipoids into the circulation." Voit (6) has shown that even where the reserve fats and lipoids enter the circulation and are carried to distant organs to be burned, as in extreme degrees of inanition, the fats and lipoids of the brain are spared, and the same has been proved to be the case in phosphorus poisoning (Mansfeld (4)).

Richet (7) called attention to the fact that the less soluble a substance is in water the more marked are its narcotic powers, and he sought to lay this down as a general law, but it is easily demonstrable that such is not the case. For example, alcohol and chloral are equally soluble in water, yet chloral is a much more powerful narcotic. Meyer (8) and Overton, working independently, attempted to show what relation, if any, existed between the solubility of a narcotic in fats and oils and its narcotic power, and later Baum (9) showed that the anesthetic properties of a drug depend not so much on its lack of solubility in water or its solubility in fats as on its distribution coefficient or relative solubility in a mixture of water and some oily or fatty substance, and that anything that changes this coefficient correspondingly alters the narcotic power of the drug.

In the animal body we have a multitude of cells composed of semifluid protoplasm and fatty bodies or lipoids. These cells are surrounded by a tissue fluid or watery lymph. If these facts are applicable to the animal body, the following postulates stated by Meyer must be true.

1. All chemically indifferent substances that are soluble in fat and fat-like substances must have a narcotic action upon protoplasm in proportion to their ability to diffuse therein.

2. The narcotic effect will appear first and most powerfully in cells in whose chemical constitution the fat-like bodies predominate and particularly in nerve cells.

3. The degree of narcosis produced by any one of these drugs depends, on the one hand upon its affinity for fat-like substances, on the other hand upon its affinity for the other cell body constituents, foremost among these water; in other words, upon its distribution coefficient as determined by its distribution in a mixture of water and fat-like substances.

[3] Cited by Meyer, H., *Arch. f. exper. Path. u. Pharmakol.*, 1899, xlii, 114.

Baum (9) determined the distribution coefficient for a number of narcotics, using a mixture of water and olive oil, and was able to show that the degree of narcotic power of any drug depends upon its distribution coefficient in this mixture. He showed further that anything that alters this coefficient, even though the chemical composition of the drug remains the same, correspondingly alters the degree of its narcotic power, and that substances having the same distribution coefficient have the same narcotic power, although their chemical composition and properties are different. With these facts in mind, Meyer and Overton independently advanced the following theory.

The narcotizing substance enters into a loose physicochemical combination with the lipoids of the cells, perhaps with the lecithin, and in so doing changes their normal relationship to the other cell constituents through which an inhibition of the entire cell chemism results.

This simple theory explains why and how the narcotic, which is a protoplasmic poison, is able to pass the semipermeable lipoid membrane, and enter the cell, but it casts no light upon the nature of the alterations in cell chemism that follow. In fact, the implication is that, since the narcotics are chemically indifferent bodies, they do not take part in the chemical processes of the cell. That the chemistry of the cell undergoes decided changes was soon demonstrated when Becker (10) first discovered acetone in the urine of patients who had been anesthetized, and that the narcotic state is dependent upon these alterations in cell chemism has been shown by Verworn (11), Mansfeld (4), Bürker (12), Heaton (13), and others.

Reicher (2) showed that the following metabolic changes are constantly present after prolonged narcosis:

(1) An increase in the total, and particularly in the ammonia nitrogen in the urine. (2) An increase in the acetone bodies in the urine and expired air. (3) An increase in the total fat of the blood as determined by extraction with ether and alcohol. This increase amounted in some cases to three and four times the normal amount. (4) An increase in the lecithin and cholesterin, the sum of which was often equal to that of the fat; in other words, there was not only a lipemia but also a lipoidemia. (5) An increase in the quantity of fatty acids in the blood.

Reicher (3) suggests that the lipemia is not a meaningless phenomenon, but, on the contrary, represents a mechanism by means of which the body cells protect themselves against the poisonous narcotic. The fat molecules which are excreted by the cells into the circulation and which have a strong affinity for the narcotic he considers as amboceptors that unite with molecules of the invading narcotic just as an antigen with its antibody, and in this way the poison is neutralized and the vital body cells are protected.

Although at first sight this assumption seems plausible, it has almost no experimental basis and Nerking (14) attempted to supply the necessary experimental proof. He narcotized various animals (dogs, rabbits, and rats) with different anesthetics, such as chloroform, ether, morphin-scapolamin, and chloral-urethan, and, either before starting the anesthesia or after the narcotic state had been established, he injected a given dose of a 1 to 10 per cent. emulsion of lecithin in normal salt solution intravenously, subcutaneously, intraperitoneally, or intraspinally, in order to show what effect, if any, the lecithin

had upon the quantity of a given narcotic that was necessary to induce a given degree of narcosis, or whether lecithin could accelerate the reaction from the anesthetic state and eliminate some or all the unpleasant after effects. As a result of his experiments he came to the following conclusions: the injection of lecithin has an undoubted influence upon the duration and after effects of anesthesia in that it shortens its duration, brings about a more rapid return of consciousness, and eliminates unpleasant after effects. Lecithin itself is a harmless agent and it exerts a beneficial effect upon the entire organism.

Nerking's work is open to objections as regards (1) dosage and (2) the depth of the narcotic state. The narcotics he administered were given by inhalation chiefly, by subcutaneous injection, by intravenous injection as in the chloral-urethan experiments, and intraspinally. It is obvious that, except by the last two methods, accurate dosage is impossible.

METHOD.

The narcotic, in the experiments to be reported, was administered intravenously. Nerking (14), Burkhardt (15), Kümmel (16), and others had already shown the practicability of this method of anesthesia, and some of them, particularly Burkhardt and Kümmel, had already used it on a number of human beings. In one series of experiments a 5 per cent. solution of ether in normal saline was used, in another a 10 per cent. solution, and in the last series a mixture was used containing one part of a 4 per cent. solution of morphin sulphate and two parts of a 20 per cent. solution of chloral hydrate. In each case the solution was put into a long glass tube graduated in five cubic centimeter divisions. To the end of this tube a rubber tube was attached, which was tied to a cannula inserted into a small tributary of the external jugular vein. The rate of flow was regulated by means of a clamp screw. To exclude the influence of the height of the liquid column upon the rate of flow, the height of this column was the same both in the control and in the lecithin experiments and the injection in each case was commenced at the same rate.

The same animal was used both for the control and the lecithin experiments, an interval of thirty-six to seventy-two hours being allowed to elapse between the experiments in order to allow the animal to recover from the previous anesthesia. In each case sterile water was used as a local anesthetic. The criteria used were: (1) the disappearance and reappearance of the corneal reflex, which was tested by drawing a fine wire across the cornea, as this will often

elicit a reflex when simply touching it will not; (2) the time when the animal raised its head; (3) the time when it first got upon its feet; and (4) the time of complete disappearance of all ataxia. Cutaneous sensation was determined by pinching the skin with dull scissors, the same part of the skin being used each time. The lecithin[4] in the form of a 5 or 10 per cent. emulsion in saline was injected directly into the vein through the same cannula as the narcotic solution.

PROTOCOLS.

SERIES I.

Experiment 1. Control.—April 3, 1912. Rabbit, black. Weight 1,200 gm. Local anesthetic, sterile water.

3.05 P. M. Injection of 5 per cent. ether solution begun. Rate 15 c.c. per minute.

3.07 P. M. Corneal reflex gone. Solution used, 25 c.c. Rate of injection reduced to 10 c.c. per minute. Respiration, 82 per minute.

3.17 P. M. Injection discontinued. Total solution used, 95 c.c.

3.21 P.M. Corneal reflex present.

3.26 P. M. Raises head and tries to get up.

3.30 P. M. Upright position readily regained when disturbed, but animal is quiet.

Lecithin.—April 7, 1912. Same rabbit. No local anesthetic.

10.35 A. M. 17 c.c. of 5 per cent. lecithin emulsion injected into vein through cannula in 3 minutes.

10.45 A. M. Injection of 5 per cent. ether solution begun.

10.47 A. M. Corneal reflex gone. Solution used, 20 c.c.

Pulse, 240. Respiration, 60.

10.57 A. M. Injection discontinued. Total quantity of solution used, 95 c.c. Total time, 12 minutes.

11.09 A. M. Corneal reflex distinct.

11.20 A. M. Raises head.

Experiment 2. Control.—April 8, 1912. Rabbit, white.

9.50 A. M. Injection of 5 per cent. ether solution begun.

9.55 A. M. Corneal reflex gone; solution used, 60 c.c.

10.00 A. M. Injection stopped. Total solution used, 105 c.c.

10.05 A. M. Corneal reflex present.

10.10 A. M. Raises head after stimulation.

10.12 A. M. Very active but cannot regain vertical position.

10.17 A. M. Still drags hind legs; otherwise normal.

Lecithin.—April 10, 1912. Same rabbit.

3.05–3.09 P. M. Injection of 20 c.c. of 5 per cent. lecithin emulsion.

3.12 P. M. Injection of 5 per cent. ether solution begun.

[4] The lecithin was obtained from Poulence Frères, Paris, from whom Nerking obtained his product.

TABLE I.

Series 1.

Animal.		5 per cent. lecithin emulsion injected.	Injection begun.	Corneal reflex gone in	Injection stopped.	Rate per minute until under anesthetic.	Rate per minute after under anesthetic.	Total solution used.	Corneal reflex present in	Head raised in	Recovered in
Rabbit 1, black, male; weight 1,200 gm.	Control		3.05 P.M.	2 min.	3.17 P.M.	15 c.c.	10 c.c.	95 c.c.	4 min.	9 min.	13 min.
	Lecithin	17 c.c. 10.35 A.M.	10.45 A.M.	2 min.	10.57 A.M.	10 c.c.	7.5 c.c.	95 c.c.	12 min.	23 min.	
Rabbit 2, white, male.	Control		9.50 A.M.	5 min.	10.00 A.M.	12 c.c.	9 c.c.	105 c.c.	5 min.	10 min.	17 min.
	Lecithin	20 c.c. 3.05–3.09 P.M.	3.12 P.M.	5 min.	3.23 P.M.	10 c.c.	9 c.c.	105 c.c.	7 min.	13 min.	22 min.

3.17 P. M. Corneal reflex gone. Solution used, 50 c.c.
3.23 P. M. Anesthetic stopped; 105 c.c. used.
3.30 P. M. Corneal reflex present but animal is still very drowsy.
3.36 P. M. Raises head but is still very drowsy.
3.40 P. M. More lively, is able to regain vertical position.
3.45 P. M. Fully recovered.

The results in table I do not indicate that lecithin administered before anesthesia is begun has any influence upon the readiness with which the narcotic state may be induced, or that it accelerates the animal's reaction from the effects of the narcotic.

SERIES 2.

Experiment 1. Control.—Rabbit, gray. Weight, 1,200 gm.
Local anesthetic, sterile water.
2.54 P. M. Injection of 10 per cent. ether solution begun. Rate, 10 c.c. per minute.
2.58 P. M. Corneal reflex gone. Solution used, 23 c.c. Respiration, 74.
3.03 P. M. Injection discontinued. Total solution used, 43 c.c.
3.06 P. M. Corneal reflex present; moves jaw, raises head.
3.12 P. M. Assumes erect position but hind legs show marked weakness.
3.20 P. M. Fully recovered.
Lecithin.—Same rabbit.
11.34 A. M. Injection of 10 per cent. ether solution begun.
11.39 A. M. Corneal reflex gone. Solution used, 25 c.c.
11.44 A. M. Injection stopped. Additional solution used, 25 c.c.
11.45 A. M. Injection of 7 c.c. of 5 per cent. lecithin emulsion into vein.
11.46 A. M. Corneal reflex present.
11.51 A. M. Moves left leg.
12.02 P. M. Tries to hop.
12.07 P. M. Fully recovered.
Experiment 2. Control.—Rabbit, gray. Weight 1,120 gm.
Local anesthetic, sterile water.
2.14 P. M. Injection of 10 per cent. ether solution begun. Rate, 10 c.c. per minute.
2.17 P. M. Corneal reflex gone. Solution used, 35 c.c.
2.23 P. M. Injection discontinued. Total solution used, 55 c.c.
2.28 P. M. Corneal reflex present; raises head.
2.31 P. M. Assumes upright position but shows marked ataxia.
2.34 P. M. Still shows incoördination on attempting to move.
2.36 P. M. Hops about. Normal.
Lecithin.—Same rabbit.
10.44 A. M. Injection of 10 per cent. ether solution begun.
10.48 A. M. Corneal reflex gone. Solution used, 35 c.c.
10.53 A. M. Injection discontinued. Total solution used, 55 c.c. 8 c.c. of 5 per cent. lecithin emulsion injected into vein.
10.57 A. M. Corneal reflex present.

TABLE II.

Series 2.

Animal.		5 per cent. lecithin emulsion injected.	Amount of solution used.	Corneal reflex present in	Head raised in	Position erect in	Recovered in
Rabbit 1, gray; weight 1,120 gm.	Control		55 c.c.	5 min.	5 min.	8 min.	13 min.
	Lecithin	8 c.c. 10.57 A.M.	55 c.c.	4 min.	15 min.	17 min.	19 min.
Rabbit 2, gray; weight 1,200 gm.	Control		43 c.c.	3 min.	3 min.	11 min.	17 min.
	Lecithin	7 c.c. 12.08 P.M.	50 c.c.	3 min.	13 min.	15 min.	30 min.
Rabbit 3, gray; weight 1,200 gm.	Control		55 c.c.	3 min.	14 min.	19 min.	24 min.
	Lecithin	10 c.c. 12.39 P.M.	50 c.c.	4 min.	14 min.	15 min.	18 min.

11.01 A. M. Lies on side, cannot raise head.
11.08 A. M. Holds up head.
11.10 A. M. Hops about but still shows ataxia.
11.12 A. M. Fully recovered.
Experiment 3. Lecithin.—Same rabbit.
12.27 P. M. Injection begun. Rate, 7 c.c. per minute. Solution used, 25 c.c.
12.31 P. M. Corneal reflex gone. Respiration, 80 per minute. Rate, 4 c.c. per minute.
12.37 P. M. Injection discontinued. Total solution used, 50 c.c.
12.41 P. M. Corneal reflex present.
12.46 P. M. Lies on side. Expression dull.
12.48 P. M. Cannot raise head.
12.51 P. M. Raises head after stimulation.
12.52 P. M. Standing, but shows signs of weakness.
12.55 P. M. Hops about. No ataxia.

In only one of the experiments (rabbit 3) given in table II does the administration of lecithin seem to have had any accelerating effect on the recovery, and this was seen during the latter stages. The other experiments show a distinct retardation.

SERIES 3.

Experiment 1. Control.—Rabbit, gray.
3.20 P. M. Injection of a 5 per cent. ether solution begun.
3.34 P. M. Corneal reflex gone. Solution used, 65 c.c.
3.39 P. M. Injection discontinued. Total solution used, 95 c.c.
3.45 P. M. Corneal reflex present; animal raised head.
3.50 P. M. Erect position.
3.55 P. M. Fully recovered.
Lecithin.—Same rabbit.
11.27 A. M. Injection commenced.
11.39 A. M. Corneal reflex gone. Solution used, 65 c.c.
11.44 A. M. Injection discontinued. Total solution used, 95 c.c.
11.46 A. M. 10 c.c. of 5 per cent. emulsion injected.
11.48 A. M. Corneal reflex present.
11.53 A. M. Fully recovered.
Experiment 2. Control.—Dog, male. 10 per cent. solution of ether used.
3.17 P. M. Injection begun. Rate, 40 c.c. per minute.
3.24 P. M. Corneal reflex gone. Total solution used, 140 c.c.
3.27 P. M. Injection discontinued. Total solution used, 170 c.c.
3.28 P. M. Corneal reflex present.
3.40 P. M. Moves about, but is ataxic.
3.45 P. M. Fully recovered.
Lecithin.—Same animal.
10.40 A. M. Injection begun. Rate, 35 c.c. per minute.
10.48 A. M. Corneal reflex gone. Solution used, 190 c.c.
10.49 A. M. Respiration ceases; artificial respiration.

10.51 A. M. Injection discontinued.
10.52 A. M. 30 c.c. of 5 per cent. lecithin emulsion injected.
10.56 A. M. Animal raises head.
10.59 A. M. Upright position; very ataxic.
11.03 A. M. Fully recovered.

TABLE III.

Series 3.

Animal.		5 per cent. lecithin emulsion injected.	Amount of solution used.	Corneal reflex present in	Head raised in	Position erect in	Recovered in
Rabbit, gray.	Control		95 c.c.	6 min.	6 min.	11 min.	18 min.
	Lecithin	10 c.c.	95 c.c.	4 min.			11 min.
Dog, black . .	Control		170 c.c.	1 min.		15 min.	18 min.
	Lecithin	30 c.c. 10.52 A. M.	205 c.c.	1 min.	4 min.	10 min.	11 min.

Series 3 shows a somewhat more rapid recovery from the effects of the anesthesia when lecithin was injected.

Morphin-Chloral Experiments.

In this series a mixture consisting of two parts of a 20 per cent. solution of chloral-hydrate and one part of a 4 per cent. solution of morphin-sulphate was used. The solution was injected slowly into a tributary of the external jugular vein, the injection of three to four cubic centimeters taking about five minutes. In each case the respirations became very slow and shallow, and in some cases ceased entirely and artificial respiration was used.

SERIES 4.

Experiment 1. Control.—August 29, 1912. Rabbit, male. Weight 3,300 gm.

3.30–3.35 P. M. Injected 4 c.c. of above solution. Immediate loss of sensation with marked reduction in sensitiveness of cornea, but no loss of corneal reflex.

3.55 P. M. Raises head after severe cutaneous stimulation.
4.10 P. M. Moves legs involuntarily.
4.15 P. M. Can regain vertical position but is very ataxic.
4.50 P. M. Still shows ataxia and cannot move even after stimulation.

Lecithin.—August 31, 1912. Same rabbit.

12.09–12.15 P. M. Injected 3 c.c. of same solution. Respiration stopped. Corneal reflex present. Sensitiveness of cornea very much reduced. Respiration, 2 per minute.

TABLE IV.

Series 4.

Animal.		10 per cent. lecithin emulsion injected intravenously.	Amount of solution used.	Insensible to pain in	First response to pain in	Head raised in	First voluntary movement in	Recovered in
Rabbit *A*, white, male; weight 3,300 gm.	Control		4 c.c.	3 min.	20 min.	20 min.	35 min.	
	Lecithin	6 c.c.						No response to pain after $1\frac{3}{4}$ hrs.
		12.30 P.M.	3 c.c.	3 min.				
Rabbit *B*, white, female; weight 2,200 gm.	Control		3 c.c.	5 min.	20 min.	20 min.	20 min.	32 min.
	Lecithin	9.5 c.c.	9.5 c.c.	2 min.	32 min.	32 min.	32 min.	44 min.

12.20–12.25 P. M. 6 c.c. of 10 per cent. lecithin emulsion injected into vein.
12.25 P. M. Corneal reflex present.
12.50 P. M. Winks eyes. No response to pain stimuli.
1.50 P. M. Sewing up wound caused no response.
2.00 P. M. Still no response to intense pain stimuli.

Experiment 2. Control.—August 29, 1912. Rabbit, female, white. Weight, 2,200 gm.

4.05–4.10 P. M. Injected 3 c.c. of same solution into tributary of external jugular vein. Respiration very slow and shallow. Corneal reflex slightly sensitive. No pain sensation.
4.30 P. M. Responds to stimulation, winks eye, raises head.
4.45 P. M. Fully recovered.

Lecithin.—August 31, 1912. Same rabbit.

1.09 P. M. Injected 0.5 c.c. of same solution. Leak found in tube, clot in cannula. Injections stopped.
1.12 P. M. Injected 3 c.c. of solution. Respiration stopped. Corneal reflex gone.
1.15 P. M. Breathing again, slowly but deeply.
1.15 P. M. 9.5 c.c. of 10 per cent. lecithin emulsion injected into vein.
1.30 P. M. Winks; corneal reflex present; no sensitiveness to pain present.
1.45 P. M. Responds to stimulation, raises head, hops about when stimulated but is very ataxic.
1.57 P. M. Fully recovered.

Series 4 demonstrates the ineffectiveness of lecithin in shortening the duration of anesthesia. In each case in which lecithin was used there was a distinct retardation in the reaction time.

From the above experiments the following conclusions may be drawn.

1. The intravenous injection of five to thirty cubic centimeters of a 5 or 10 per cent. emulsion of lecithin, depending upon the size of the animal used, does not interfere with the induction of anesthesia, and this can be accomplished as readily in animals thus injected as in controls.

2. In six out of nine experiments lecithin had no effect upon the rapidity with which the various phenomena which indicate the animal's recovery from the effects of the anesthetic appeared.

The above experiments do not bear out Reicher's assumption as to the cause of the lipoidemia, and the explanation of this phenomenon still remains an open question.

In conclusion, I desire to express my thanks to Dr. John R. Murlin for suggesting the subject and for his assistance, and to

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VARIATIONS IN A CHICKEN SARCOMA CAUSED BY A FILTERABLE AGENT.*

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Plates 41 to 49.

Marked alterations in structure and behavior are not uncommon in tumors which run their course in a single individual; but, as might be expected, they are more frequent and better marked in growths of which the existence is prolonged by transplantation. The present paper has to do with the changes that have taken place in a spindle-celled sarcoma of the fowl[1] during its propagation for a period of about three years, and in numerous individuals. The findings have especial claim to attention because the sarcoma, otherwise a typical tumor, can be transferred to new hosts, not only by transplantation, but by means of an agent which is separable from the neoplastic cells, and is probably a living organism. Possibilities of variation are thus introduced which do not appear in the case of tumors that depend for their transmission solely upon the survival of a parent strain of cells.

In several previous communications, the characters of the chicken sarcoma have been taken up in detail.[2] Under ordinary circumstances its growth takes place by a proliferation of the cells already neoplastic, and apparently in that way only; while the metastases are referable entirely to autotransplantation. The transmission of the disease to other susceptible hosts is most readily accomplished by implanting in them a bit of the sarcomatous tissue, which, as experiment has shown, will survive in the new site, and by its proliferation give rise to the new tumor. The growth is at present developing in

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[1] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

[2] Rous, P., *loc. cit.*; Rous, P., and Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lviii, 1938; *Jour. Exper. Med.*, 1912, xv, 270; Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751, 1840.

the thirty-second successive series of fowls to which it has been thus transferred. Despite our knowledge that the individual neoplasms arise practically *in toto* through a proliferation of the cells introduced from the last host, it is uncertain whether the present tumors are strictly the offspring by cell division of the original growth. For a transforming action of the causative agent upon normal tissue which would be negligible in the individual case might well bring about in the course of time and many transplantations a total change in the growth's make-up. At all events the tumors of the thirty-two series of transplantations represent the most direct possible continuance of the original sarcoma strain. In addition, many growths have been studied which were produced by the causative agent acting alone, either as a filtrate or as dried or glycerinated tumor tissue, and many more which may have resulted either from an action of the agent or the survival of tumor cells, since they developed after the implantation of sarcomatous tissue damaged *in vitro* but not killed. In all, the primary and secondary growths of 217 fowls have been carefully gone over.

LOCAL VARIATIONS.

The structural variations occurring under ordinary conditions in different parts of the same sarcoma will first be taken up. Certain of these, due to degeneration or to attempts at differentiation, are found in nearly every specimen.

A typical growth consists of spindle cells, more or less attenuated, coursing this way and that in irregular strands. When rapid proliferation is taking place the cells near the border of the growth may be short and blunt, or even irregularly rounded (figure 1). As the mass increases in size and the cells once at its edge come to lie deep within it, they cease to divide so actively, gradually assume an attenuated spindle form, and produce intercellular fibrils. A similar progressive differentiation is often noted in the tumors of human beings.

For the most part the active cells in the best nourished portions of the sarcoma are the ones which enter the blood vessels and are distributed to distant parts (figure 2). The secondary foci partake of the character of the emboli from which they arise, and they may

consist, when small, entirely of active cells of blunt or rounded form (figure 3), appearing for this reason markedly different from the older tissue of the primary mass. But as time passes and the metastases enlarge, their cells gradually become spindle-shaped like those in the parent growth. In many instances the metastases consist from the beginning of spindle cells.

Degenerative changes are of several sorts. In old tumors the spindle cells may become extremely attenuated (figure 4), if necrosis does not overtake them. More frequently cell death occurs early, often preceded by a hydropic change. In the latter case the cells may become greatly swollen and multinucleated. A myxomatous change is not infrequent, and is seen especially at the center of fairly nourished, slowly growing tumors in resistant hosts. It cannot be looked upon as intermediate to the ordinary necrosis.

Very striking accommodative changes are sometimes seen. Their dependence on special conditions of pressure and nutrition in the organ containing the tumor is usually very evident. In cross section growths in voluntary muscle have often an alveolar structure,[3] the result of replacement of the muscle fibers with retention of their outline. Growths in the lungs may present a very complex picture. The pulmonary air spaces may contain masses of actively dividing spheroidal tumor cells, easily distinguishable from desquamated epithelium; whereas nearby in the interstitial tissue the neoplastic cells are of spindle form in strands (figure 5). The structure of the ovary leads to a somewhat similar diversity there. In the liver, kidney, spleen, and heart the sarcoma is evenly constituted of spindle cells. In the gizzard, an organ of very close texture, the growth is often non-invasive, and its spindle cells are compactly arranged and often are attenuated. All these various pictures may be seen in the metastases of a single tumor. It is not improbable that pressure changes have something to do with the gradual development of the attenuated spindle form in the older portions of primary growths.

Certain striking histological differences are sometimes observed in secondary tumors seated close to one another in the spleen or liver. Of two neighboring foci in the liver one may consist of sharply defined, rounded, or even cuboidal cells, arranged in columns within

[3] Rous, P., *Jour. Exper. Med.*, 1911, xiii, 397.

the portal spaces, and superficially very suggestive of newly formed bile ducts; while the other has the usual spindle-celled form. In the spleen there are sometimes found, in addition to nodules of the ordinary sarcoma, sharply circumscribed growths consisting entirely of short, closely crowded, fusiform cells (figure 6) in which mitoses are unusually numerous.

At first sight it would seem unlikely that these diverse secondary nodules are true metastases of a single tumor. Instead it might be assumed that they have arisen in part by localizations of the sarcoma-producing agent in connective tissue cells of unusual potentiality, lying within the affected viscus. But against this assumption are our experiments which show that the agent rarely if ever acts to cause secondary nodules,[4] and the direct proof that these latter are in general the result of a dissemination of cells from the primary growth. Furthermore all morphological gradations can be traced between the ordinary sarcoma and the peculiar tumors, and as the latter enlarge, their cells take the spindle form. When one considers how markedly local differences in nutrition and pressure influence the appearance of the sarcoma, and the fact that the histology of each beginning metastasis depends to some extent on the character of the embolus from which it has arisen (embolus of rounded cells, embolus of spindle cells), the assumption of a secondary localization of the agent seems unnecessary. On the other hand, the occasional occurrence of such a localization can not be absolutely ruled out.

CHANGES WITH INCREASED MALIGNANCY.

Some of the most marked changes in the sarcoma have accompanied its increase in malignancy. The original tumor was a discrete, fairly encapsulated mass, firm and gristly, with a striated cut surface. At its center was an irregular core of coagulation necrosis. The tumors of the first few generations were quite similar, but often contained one or more cysts full of pale yellow, mucinous fluid. With the great increase in transplantability and rapidity of growth which took place between the second and sixth tumor gener-

[4] Rous, P., and Murphy, J. B., *Jour. Exper. Med., loc. cit.;* Rous, P., Murphy, J. B., and Tytler, W. H., *loc. cit.*

ations, the character of the neoplastic tissue altered markedly. It became soft, friable, and translucent, with little striation and no encapsulation. Rapid breaking down *en masse* occurred often, and in these cases amyloid was sometimes found in the spleen and liver.[5] Hemorrhage into the tumor substance with the formation of large, ragged cavities full of brown or green or bloody fluid was very frequent. The cells had often a very irregular spindle form, or were short and blunt or even rounded. Their increased activity is perhaps best illustrated by the change in their behavior toward striped muscle. In the earlier generations they had supplanted this by penetrating in strands between its fibers, bringing about their gradual atrophy and disappearance; but now they often apposed themselves directly to the fiber substance, which disappeared before them as though eroded; or else they penetrated the sarcolemma at one or more points and proliferated and extended rapidly within it[6] in a manner met with in only the most malignant of mammalian growths.

The gross form of the neoplasm was much altered with the increase in malignancy, as would naturally follow from its greater invasive power. The earlier series of growths resulting from implantation in the pectoral muscles were discrete, and frequently projected sharply from the body contour. But the later ones at this site have been much less definite in form, though still considerably firmer than the normal tissue and easily distinguished on palpation. They may first become evident to the naked eye as diffuse swellings which little by little gain in prominence through expansive growth and the emaciation of the host. Only in somewhat resistant fowls is the very discrete character of the original attained. In these the sarcoma may be encapsulated and very firm, owing to the large amount of non-neoplastic, fibrous tissue about and within it. No better example of tumor malignancy as affecting tumor form could be desired. The course of a growth in the individual fowl may be predicted with considerable accuracy from the findings on a single palpation.

[5] For amyloid changes in the organs of mice with tumors, see Lubarsch, O., *Centralbl. f. allg. Path. u. path. Anat.*, 1910, xxi, 97.

[6] Rous, P., *Jour. Exper. Med.*, 1911, xiii, 397.

INTERCURRENT VARIATIONS.

By selective transplantation the sarcoma has been kept for many months in a highly malignant state. From time to time without evident cause changes in its morphology have manifested themselves. In some hosts the tumor consists of spindle cells much smaller than in other fowls of the same age and variety; and in some the growth closely approaches in appearance a round-celled sarcoma (figure 7) though many of its cells have protoplasmic tails or queues, and there is always some differentiation to the spindle form. Most striking are the giant-celled growths.

Giant cells were numerous in some portions of the original tumor, as appears from sections of it recently made (figure 8). Nevertheless, in the nodules resulting from transplantations into the original host they were so few that the impression given was that of a practically pure spindle-celled sarcoma. In the subsequent tumors in other hosts, they were for a long time met with only infrequently and these later growths may well be called pure, spindle-celled sarcomata. But in the nineteenth transplantation generation, giant cells again appeared as an important constituent of the tumor and growths characterized by large numbers of them have since been frequent.

In appearance the giant cells (figure 9) are entirely different from those occurring about foreign bodies in the fowl or associated with avian tuberculosis. They are not arranged in foci but lie scattered among the neoplastic spindle cells, from which their development by a process of enlargement, accompanied sooner or later by degeneration, can be directly traced. They may reach a diameter of 100 microns or more, and are usually oval, with one or two blunt processes, or forks, and a single large nucleus, features which give them a superficial resemblance to ganglion cells. At first the cytoplasm is finely granular and faintly basic, as is that of the spindle cells round about; but later, as the cell enlarges, the cytoplasm stains with eosin and may become vacuolated. The single nucleus, eccentrically placed, is sometimes pyknotic, but more often vesicular and swollen, with its chromatin in a central mass (fish eye nucleus). In the smaller cells mitosis may take place, and in the larger ones

nuclear budding is frequent. Specimens are not rare with from two to forty scattered nuclei of remarkably various size and appearance. Phagocytic activity of the giant cells is frequent. Erythrocytes, leucocytes, or even small tumor cells may be ingested by them. They may be abundant in the best nourished portions of the sarcoma, indeed at its advancing edge (figure 10). They are present, as a rule, in the metastases from primary growths containing them.

The significance of the giant elements is no clearer than in the case of the sarcomatous giant cells of mammals and the conditions which lead to their appearance have not yet been recognized. In stained preparations or under the dark-field microscope they show no distinctive inclusions. During a long period they were observed only in the sarcomata which resulted from transplantation, as distinguished from those engendered by the filterable agent; but of late some change must have taken place in the agent, since now growths caused by it not infrequently contain giant cells. That peculiarities of the host have a considerable determining influence is shown by the fact that a single lot of causative virus, in the form of a filtrate or of dried or glycerinated tumor tissue, will give rise in some fowls to a pure spindle-celled growth, in others to the giant-celled form. In general the giant-celled form is relatively slowly growing, yet in hosts with an evident, though partial, resistance, tumors of either sort may be found. All things considered it would seem probable that the giant cells represent a perversion which may take place in any of the neoplastic spindle cells when under the influence of special conditions. Some of these conditions are supplied by the host, some by the growth's causative element, and others, doubtless, by local circumstances. The cells belong to the second group of giant cells in sarcomata distinguished by Mallory;[7] namely, to the group of giant cells arising out of tumor cells, in contradistinction to those developing in the non-neoplastic stroma.

HEMORRHAGIC FORM OF THE SARCOMA.

Hemorrhage in association with the sarcoma first occurred in the eighth tumor generation, some fifteen months from the time of the original transplantations. The growth had attained its max-

[7] Mallory, F. B., *Jour. Am. Med. Assn.*, 1910, lv, 1621.

imum malignancy in the sixth generation. The first bleedings that were noted took place into areas of necrosis at the center of tumor nodules, and were of slight extent and sharply localized. In the ninth and tenth generations several fowls died suddenly of hemorrhage from visceral nodules and this cause of death has since been frequent. The findings at autopsy are remarkable.

The host is nearly always one in which the tumor has grown rapidly and disseminated widely, though, because of the premature termination of the disease, the secondary growths may have had little chance to develop, and emaciation may be but slight. The lung and heart tumors are seldom hemorrhagic. Bleeding in the occasional metastases in the spleen is kept within bounds by the stout capsule of that organ. Extensive hemorrhage in the primary tumor may result in much breaking down there; but the fatal bleeding usually takes place from a liver metastasis, rarely from a focus in ovary or kidney. At autopsy the peritoneal cavity is found to be more or less distended with blood clot, arranged often in layers of different ages and degrees of firmness; and when this clot is lifted it is discovered to have a direct connection with one or several ruptured blebs or hemorrhagic tumor masses on the surface of one of the organs mentioned (figure 10).

The liver substance may be riddled with discrete, spherical cavities up to one centimeter in diameter, filled with fresh or old blood clots, which, reaching the surface, take the form of blebs. That the tumor cells have in some way caused the hemorrhage is usually evident. In one liver all gradations may be found from microscopic foci of the sarcoma in which hemorrhage has just begun, to large blood cysts which show tumor cells in only a relatively small area of their wall, or to large tumor masses which show relatively small hemorrhages in their interior. Not a few of the blood cysts have a zone of pressure necrosis about them without demonstrable tumor; but the absence of neoplastic tissue under these circumstances is scarcely surprising.

When hemorrhage takes place into the sarcomatous ovary it often converts a number of the follicles into large blood cysts, through the rupture of which death may come about. The hemorrhages in the kidney are usually small and well circumscribed, but they may

dissect into the surrounding tissue and be fatal. The intravenous injection into a normal fowl of the tumor-producing agent in the form of a Berkefeld filtrate has led in one instance to the development of a small sarcoma in the kidney, with a dissecting hemorrhage from which the fowl died.

Although the relationship between tumor and hemorrhage can scarcely be questioned in these cases, there are instances in which it is not so evident. Multiple hemorrhages in the liver of the chicken may undoubtedly be due to other causes than the sarcoma. Occasionally we have found them in the fatty liver of fowls which had not been inoculated with the sarcoma but had died of some other disease. In inoculated hosts dying of a large pectoral sarcoma but having visible metastases only in the lungs they are fairly frequent. In these instances, microscopic search usually brings out the presence of minute patches of tumor in association with some at least of the hepatic hemorrhages (figure 11), and there seems little doubt but that all should be attributed to sarcomatous foci, destroyed for the most part by the bleeding they have induced.

The structure of the growths provoking hemorrhage is often somewhat different from that of the ordinary sarcoma. The cells are short and blunt or nearly spherical, and lie loosely grouped; intercellular fibrils are few, and there is little supporting stroma. These circumstances appear to favor a wide extravasation once the rupture of a vessel has occurred. Vessels are not especially abundant, as they are in the hemorrhagic adenocarcinomata of mice, but the tumor cells are evidently invasive and make their way into many of the blood channels.

DISTRIBUTION OF THE METASTASES.

One other alteration in behavior observed during the routine propagation of the growth deserves brief mention, namely, the change in the distribution of the metastases. Elsewhere this subject has already been dealt with to some extent.[8] In the earlier generations of the tumor, the heart was most frequently affected after the lungs. In the later ones the heart has been relatively free from secondary growths and the liver has very often shown them. During the fall

[8] Rous, P., *Jour. Am. Med. Assn.*, 1910, lv, 1805.

and winter months metastases in the ovary have been rare, but now for two successive years they have been a feature of autopsies performed during the spring and summer (figure 12), or, in other words, during that period when the ovary is functioning actively, when trauma in the organ is of daily occurrence, and when its blood supply is greatly increased. The same factors have not infrequently been observed to determine secondary localizations of tumors of the mouse or man, and they may well be effective in the present case. There is some evidence to show a seasonal localization of the chicken sarcoma in the testicle as well as in the ovary, but our observations on this point have been few, since female birds have in general been used as hosts.

SARCOMATA CAUSED BY THE FILTERABLE AGENT.

Taken together, the sarcomata produced by direct injection of the filterable agent do not differ from those resulting from the growth of a bit of the transplanted sarcomatous tissue; and they manifest the same variations. Peculiarities of the individual host are important in determining these. A single lot of agent, in the form of dried, or glycerinated, tissue or a Berkefeld filtrate, will produce in some hosts a pure spindle-celled sarcoma, in others a giant-celled growth, and in yet others a growth composed of fusiform or rounded cells, and perhaps hemorrhagic. All are malignant tumors of the connective tissue, grading into one another histologically.

Attempts to obtain growths of unusual morphology by bringing the agent into contact with special types of cells have as yet been unsuccessful, and so too with attempts to obtain modifications in tumor form by attenuation of the agent *in vitro*. The growths caused by the treated agent take a long time to appear, and frequently retrogress, but otherwise they are not peculiar. The same is true of the sarcomata arising after the implantation of sarcomatous tissue which has been damaged by heat or by saponin. That the tumor cells fail to undergo a structural modification under these influences is scarcely surprising for heating does not alter the structure of transplantable mouse tumors.[9]

[9] Loeb, L., and White, E. P. C., *Centralbl. f. Bakt., 1te Abt., Orig.*, 1910. lvi, 325.

DISCUSSION.

The variations manifested by the chicken sarcoma are not more striking than certain ones which have been noted in rat and mouse tumors (Bashford, Lewin) and which are due without doubt to intercurrent changes in a single strain of tumor cells. Lewin, for example, describes an adenocarcinoma which within twelve generations assumed the form of a carcinoma solidum, a carcinoma alveolare, and a cancroid. The various forms of the chicken tumor are all sarcomata which grade into one another histologically. It would be unnecessary to suppose for them any other cause than intercurrent changes in a single strain of tumor cells were it not that an etiological agent for the tumor has been found,—an agent which accompanies the growth at all its stages, and is capable of causing, under special conditions, a neoplastic change in tissue hitherto normal. The presence of this agent leads one to ask whether the variations in the chicken sarcoma are not due in part at least to changes in it, or perhaps to its action on cells of unusual type or potentiality.

The latter possibility can be almost ruled out. Our many experiments to bring about an action of the agent on a tissue other than those it usually affects have uniformly failed. For example, when the agent, as a Berkefeld filtrate, is injected intravenously into fowls with induced lesions of various tissues the resulting tumor always arises from the spindle cells of connective tissue. On the other hand, there is no doubt but that changes in the causative agent lead to some modifications in the sarcomata of which it is the cause. When the agent is attenuated by heat, it gives rise to tumors which grow slowly and retrogress frequently. And whereas formerly the tumors caused by the agent were all spindle-celled sarcomata, now it not infrequently engenders giant-celled growths. Many of the variations in the transplantation sarcomata, expressing themselves as morphological changes or otherwise, may well be due to modifications in the agent under the play of conditions in successive hosts. The question as to whether the transplantation sarcomata observed during the last three years have consisted entirely of descendants of the original strain of tumor cells or are in part the result of a

neoplastic transformation in successive hosts does not affect this matter.

SUMMARY.

Variations are described which have from time to time occurred in the structure and behavior of a transplantable, spindle-celled sarcoma of the fowl, a growth caused, as elsewhere shown, by a filterable agent. Of late the growth has frequently given rise to fatal hemorrhages from its substance. In some of the recent, rapidly growing tumors the cells have tended to be spherical, showing only a very tardy and imperfect differentiation to the spindle form. A giant-celled form of the growth is sometimes met with. Despite their diversity the tumors grade into one another and in the final analysis are all to be considered as spindle-celled sarcomata. Attempts to obtain an action of the etiological agent upon cells other than those it usually affects have failed, as have attempts to bring about changes in the histology of the sarcomata by attenuating the agent.

Some of the lesser morphological variations in the sarcoma are undoubtedly due to local conditions in the host, and of the more important changes some have been associated with an increase in the growth's malignancy. For others the determining conditions have yet to be discovered. On the whole the variations described are not more marked than those occasionally manifested by the transplantable mammalian tumors, and traceable to the changes in a single strain of tumor cells during their propagation in successive hosts. In mammals the ultimate reason for these changes is not known. In the case of the chicken tumor some of them are undoubtedly the expression of changes in the growth's causative agent.

EXPLANATION OF PLATES.[10]

PLATE 41.

FIG. 1. Marginal portion of a sarcoma which is replacing voluntary muscle. The cells at the edge of the growth (to the left of the picture) are for the most part oval or rounded; those further in are definitely spindle-shaped. The replacement of the muscle fibres has resulted temporarily in an alveolar structure. m = muscle fibres in process of disappearance; r = rounded tumor cells.

[10] All the microscopic specimens were stained with methylene-blue and eosin

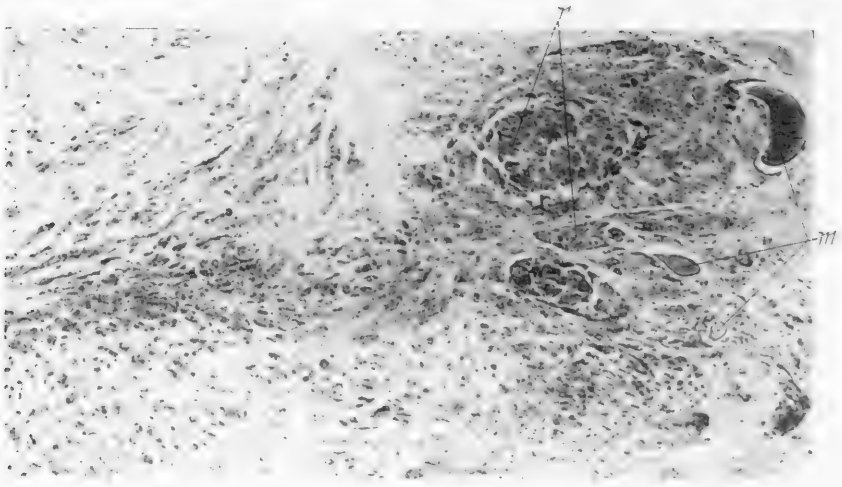

FIG. 1.

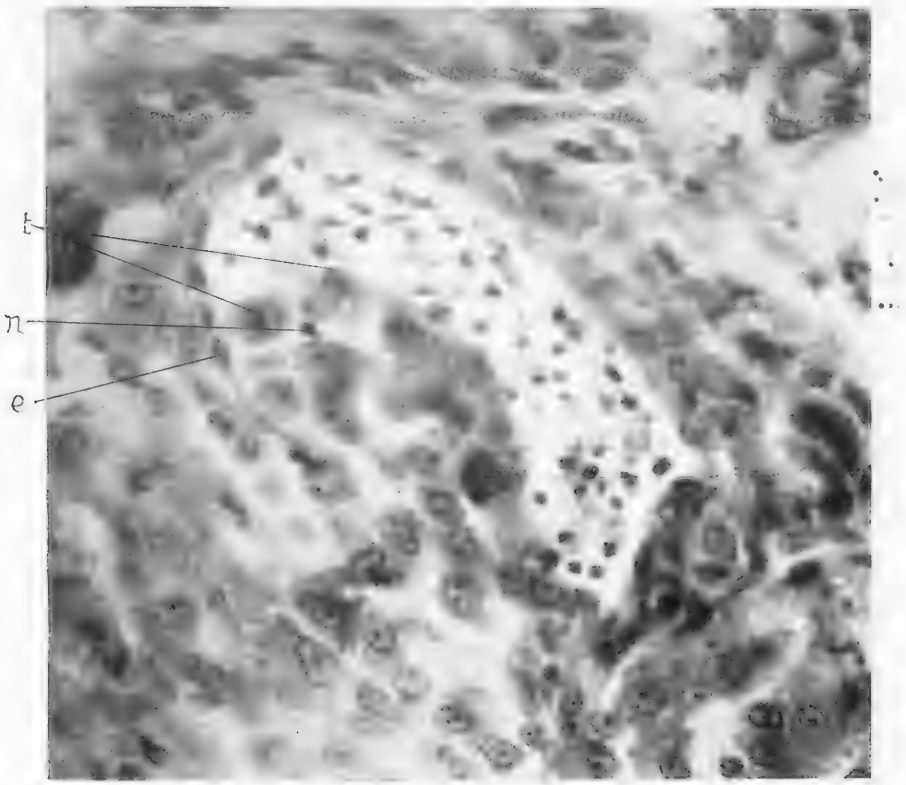

FIG. 2.

(Rous and Murphy: Variations in Chicken Sarcoma.)

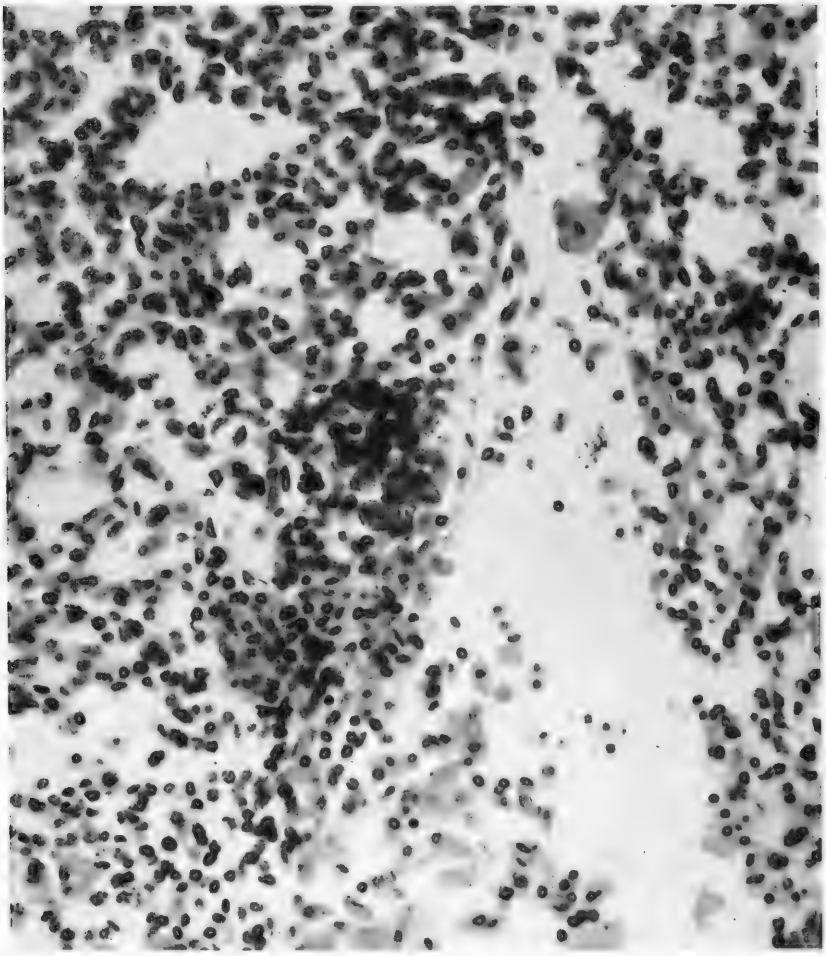

FIG. 3.

(Rous and Murphy: Variations in Chicken Sarcoma.)

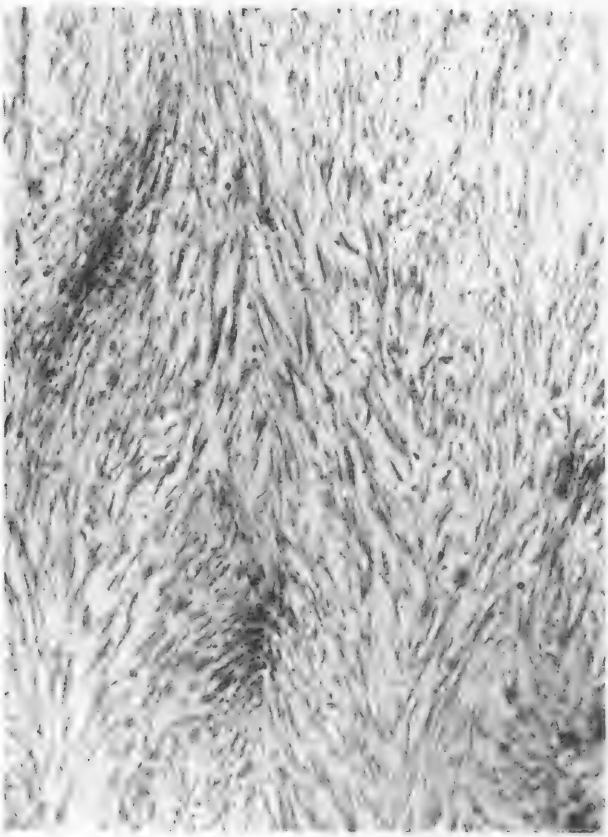

FIG. 4.

(Rous and Murphy: Variations in Chicken Sarcoma.)

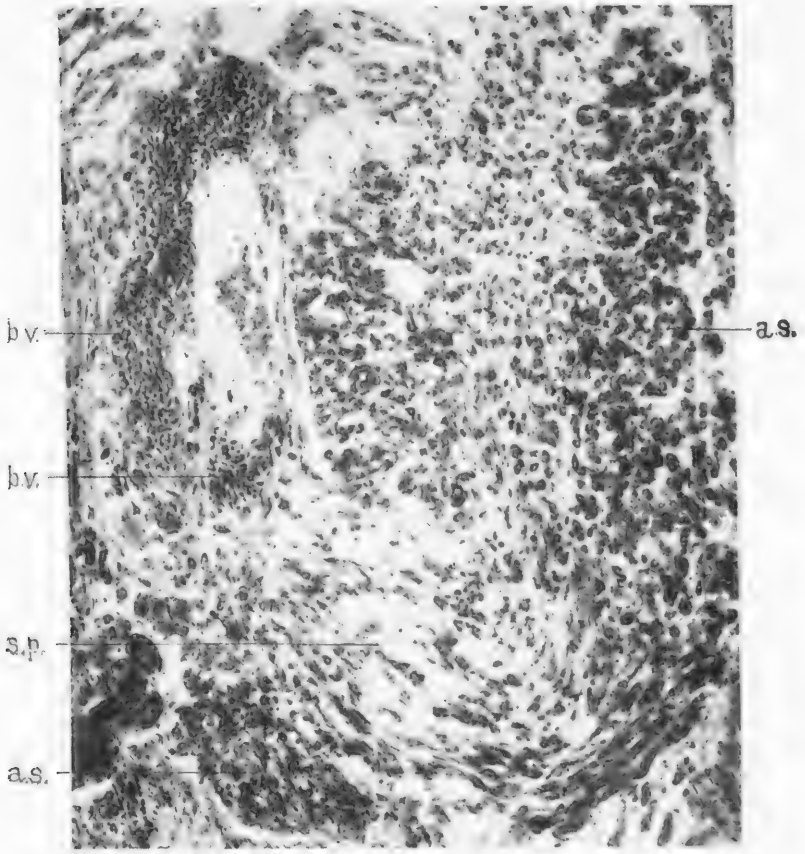

FIG. 5.

(Rous and Murphy: Variations in Chicken Sarcoma.)

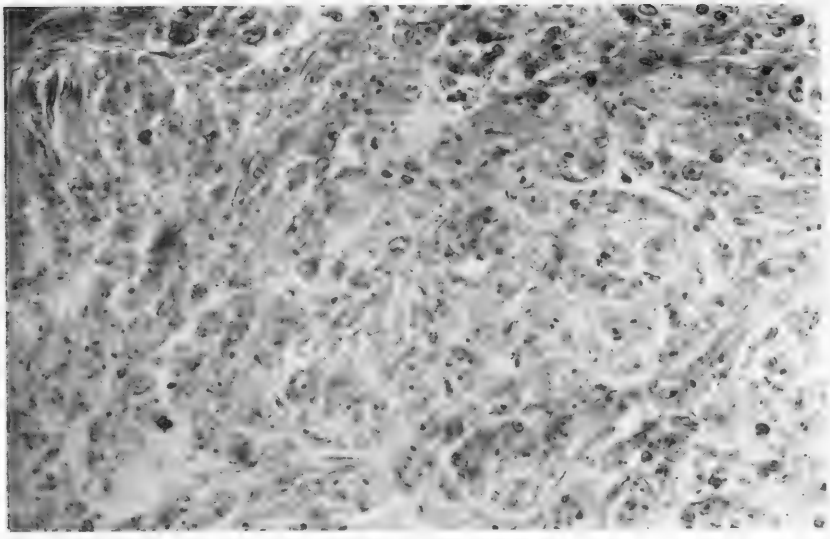

FIG. 6.

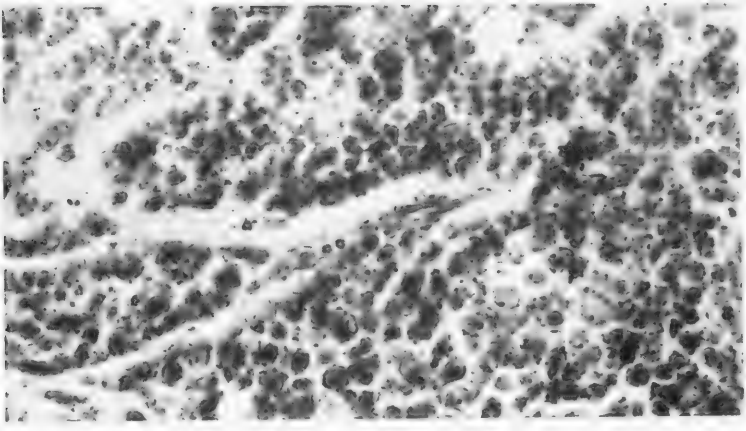

FIG. 7.

(Rous and Murphy: Variations in Chicken Sarcoma.

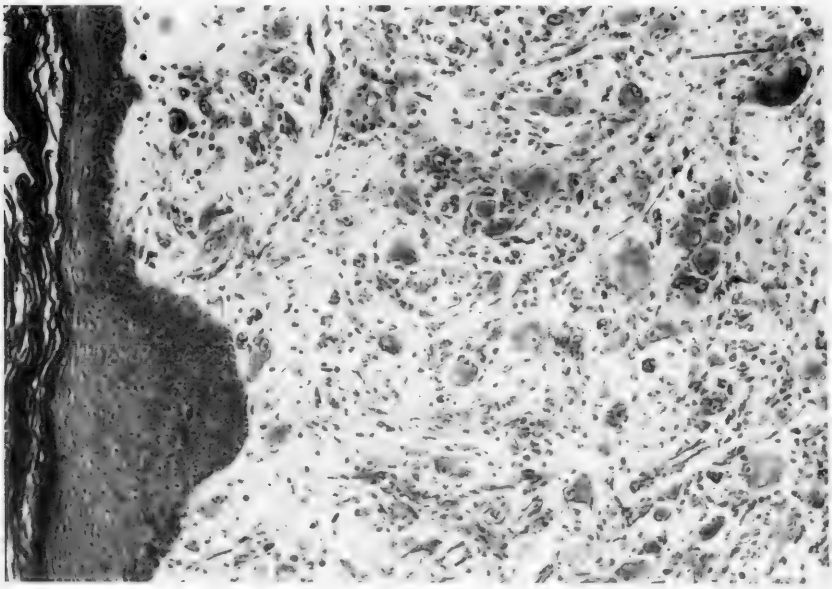

FIG. 8.

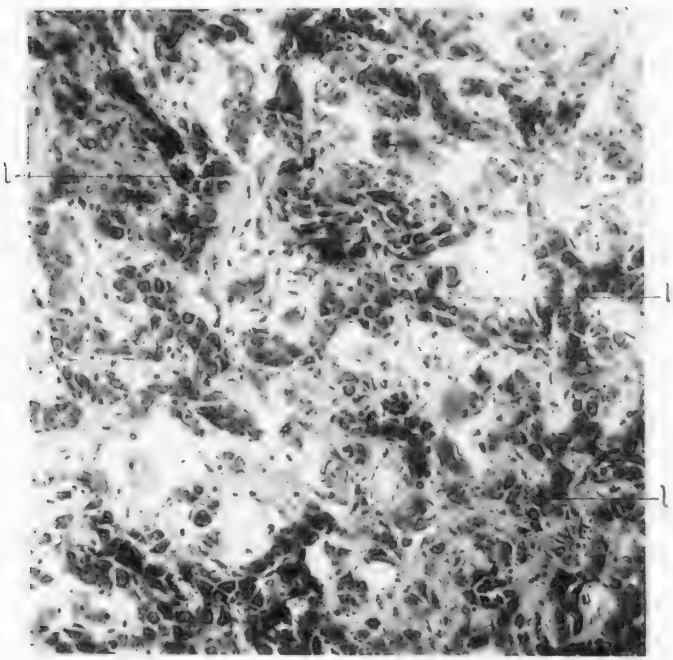

FIG. 9.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 10.

(Rous and Murphy: Variations in Chicken Sarcoma.)

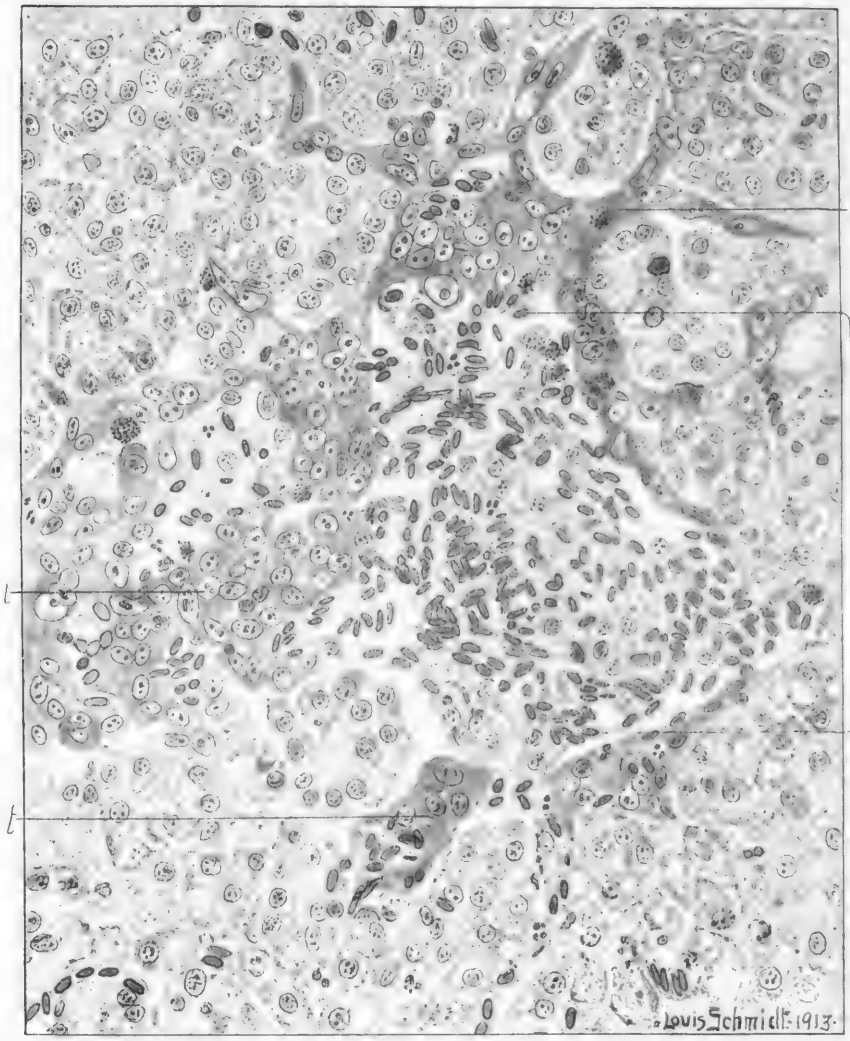

FIG. 11.

(Rous and Murphy: Variations in Chicken Sarcoma.)

CORNELL

FIG. 12.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 2. Growth of the sarcoma into the lumen of a blood vessel, showing the rounded type of tumor cell frequently cast off into the blood stream. The vessel is full of nucleated erythrocytes. *e* = endothelial lining of the vessel; *t* = tumor cells loosened from the main mass; *n* = nucleated red cell lying between the tumor cells.

PLATE 42.

FIG. 3. Colored photograph of a small metastasis in the lung. It consists of rounded cells similar to those in figure 2. Their staining reaction sharply distinguishes them from the pulmonary tissue.

PLATE 43.

FIG. 4. Attenuated spindle cells of the older portions of the chicken sarcoma.

PLATE 44.

FIG. 5. Portion of a diffuse metastasis in the lung. *b.v.* = vessels filled with nucleated erythrocytes; *a.s.* = rounded cells in the air spaces; *s.p.* = spindle-shaped tumor cells in the pulmonary tissue.

PLATE 45.

FIG. 6. Peculiar type of tumor tissue occurring in some metastases of the spleen.

FIG. 7. Portion of a sarcoma the cells of which are nearly spherical. In deeper portions of the same tumor the cells had well marked queues or tails, or were of blunt spindle shape.

PLATE 46.

FIG. 8. A section of the original sarcoma, showing tumor giant cells and infiltration of the skin.

FIG. 9. Invasion and replacement of liver tissue by sarcoma of the giant-celled type. *l* = columns of liver cells.

PLATE 47.

FIG. 10. Hemorrhage from secondary nodules in the liver. The clot partially overlies the nodule from which the bleeding occurred. Some of the others are hemorrhagic.

PLATE 48.

FIG. 11. Drawing of a minute metastasis in the liver, with a hemorrhage in its midst. *t* = tumor tissue; *h* = hemorrhage (nucleated erythrocytes).

PLATE 49.

FIG. 12. Metastases in the ovary. The sarcomatous masses hang by pedicles like true ova.

A DEMONSTRATION OF TREPONEMA PALLIDUM IN THE BRAIN IN CASES OF GENERAL PARALYSIS.*

By HIDEYO NOGUCHI, M.D., and J. W. MOORE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, and the Central Islip State Hospital, New York.*)

Plate 50.

In the present communication we wish to report the results of examinations for *Treponema pallidum* on seventy paretic brains. One of us (Noguchi) succeeded in finding the pallidum in twelve out of the seventy specimens.[1]

The relationship of paresis to syphilis has, for years, been one of the foremost topics of medical interest, especially to psychiatrists. Since 1857 when attention was first called[2] to the frequency of syphilis in the history of patients with paresis, the etiological importance of the former disease has steadily gained recognition, until now probably the majority of writers agree with Kraepelin[3] that "We can to-day declare with the greatest certainty that syphilitic infection is an essential for the later appearance of paresis." Many, however, including a man of such enormous experience as Nonne, still refuse to concede that syphilis is more than an extremely common causative factor. Nonne[4] says, "At the outset I desire to make it clear that progressive paralysis is not a specific syphilitic disease of the brain."

Among those who hold "without syphilis, no paresis," there are some who contend that paresis is nothing more nor less than a particular form of tertiary syphilis. Kraepelin objects to this view

* Received for publication, January 11, 1913.

[1] The findings were confirmed later by Dr. Moore as well as by others, among whom we may mention Dr. Flexner and Dr. Dunlap. We take pleasure in expressing our gratitude to Dr. G. A. Smith, Superintendent of the Central Islip State Hospital, for the material studied.

[2] Esmarch, F., und Jessen, W., *Allg. Ztschr. f. Psychiat.*, 1857, xiv, 20.

[3] Kraepelin, E., Psychiatrie, Leipzig, 1904, ii.

[4] Nonne, M., Syphilis und Nervensystem, Berlin, 1902.

on two principal grounds,—the distinctly greater interval between syphilitic infection and paresis as compared with that in cerebral syphilis and the refractoriness of paresis to antisyphilitic treatment This author, however, in commenting on the hitherto vain attempts to find *Treponema pallidum* in the tissues and body fluids of paresis, remarks, "This does not mean that the spirochæta is never present in the body of the paretic. It may have assumed forms so far unknown to us or have located in places where it has not yet been sought or where it is hard to find."

Of late years a few cases have been reported in which the findings of paresis occurred along with those of cerebral syphilis. Sträussler[5] has recently added two cases of this combination to several he had already reported. Ranke[6] and others have demonstrated *Spirochæta pallida* in the pia and vessel sheaths in congenitally syphilitic brains. In this country Dunlap, of the New York State Psychiatric Institute, has also shown the organisms in a case of cerebral syphilis. Dunlap has long been of the opinion that, although tertiary syphilis of the brain and general paralysis are quite distinct and seldom, if ever, occur together, they are but different manifestations of the same process.

The failure up to the present time to discover *Treponema pallidum* in the affected nervous system has doubtless added to the general conception that paresis, although of syphilitic origin, can exist without the pallidum playing an active part in the progressive processes. This assumption is valid, of course, only when we grant that *Treponema pallidum* can infallibly be demonstrated by the microscopical techniques hitherto employed. But, as will be seen presently, this is far from being the case. Besides, the individual experience on the part of the examiner has much to do with whether the organisms are viewed or remain undetected, especially in a tissue in which so many tortuous fibers are present as in the brain.

These facts, coupled with the very significant serological similarities between syphilis and paresis, and the fact that the trypanosome

[5] Sträussler, *Ztschr. f. d. ges. Neurol. u. Psychiat., Orig.*, 1912, xii, 365.

[6] Ranke, *Ztschr. f. d. Erforsch. u. Behandl. d. jugendl. Schwachsinns*, 1909, ii, 32, 81, 211.

can be found in the brain in sleeping-sickness,—a disease in many respects similar to paresis,—led the writers to believe that further search for the syphilitic organism, either in granular or in spirochætal form,[7] in paresis was warranted. To this end, tissues were taken from seventy paretic brains in the possession of the pathological service of the Central Islip State Hospital and stained with the Levaditi silver method modified in certain respects to produce an elective stain for the pallidum. The specimens in most of the cases were taken from the first right frontal gyrus; in some, from the left hemisphere or gyrus rectus.

The syphilitic organisms were found in twelve cases which were examples of undoubted general paralysis. The cases in which the pallida were found showed the classical physical signs. The post-mortem findings in the brain in every case were definitely those of general paralysis. Ten were men and two were women. Seven were of the cerebral type, and five of the tabetic. In several instances the patients had been picked up on the street in a confused state and had no idea of the duration of the condition. In the seven cases in which the onset could be satisfactorily determined, the average duration was seventeen months, the longest thirty months, and the shortest five months. The majority were much below the average duration, as estimated by various authors, which varies from twenty-four to thirty-two months. It is possible, then, that we are more apt to find the spirochæta in those cases which run a fairly rapid course. The age varied from thirty-three to sixty years, with an average of forty-four years. Brief abstracts of the case records follow.

J. C. (103), age fifty years; duration of disease unknown. He was committed from the workhouse. There was much confusion; he was disoriented and his memory was extremely poor. The knee-jerks were unequal and exaggerated; the pupils sluggish and unequal. His speech was distorted, and there were marked tremors. Convulsions occurred. Death occurred after seven months in the hospital.

C. M. (106), age thirty-nine years; duration unknown, but probably about four months. Syphilis ten years previously. When he was admitted he was depressed and apprehensive; he had hallucinations of hearing; he was disoriented, and his memory was very poor, with spells of marked confusion. The

[7] Noguchi, H., *Jour. Am. Med. Assn.*, 1912, lix, 1236.

knee-jerks were normal, the pupils sluggish. There were ataxia, distorted speech, coarse tremors, and convulsions. He died after eleven months in the hospital.

F. B. (113), age thirty-six years; duration before admission, fourteen months. Syphilis was denied. On admission he was dull, silly, mildly restless, and had occasional hallucinations. His memory showed marked discrepancies. The knee-jerks were absent, and the pupils slow to light and unequal. His speech was distorted, and he had convulsions. Later he developed a silly elation and deteriorated progressively. Death occurred after fourteen months in the hospital. The total duration of the disease was thirty months.

S. V. (131), colored, age thirty-seven years; the duration of the disease before admission is unknown. Syphilis eight years previously. He was euphoric, his memory was very poor, and he was disoriented. The knee-jerks were much diminished, the pupils slightly slow, his speech ataxic, and there were coarse tremors. Later he developed typical expansive ideas and became very ataxic. Death occurred after twenty-seven months in the hospital.

J. D. (138), age thirty-three years; duration before admission, five months. He was admitted in an hallucinatory depression with nihilistic ideas and a history of a probable attempt at suicide. A complete mental status was impossible. The knee-jerks were exaggerated; pupils unequal, Argyll-Robertson sign present; there were marked tremors. The spinal fluid showed marked lymphocytosis. Death occurred after ten months in the hospital.

F. M. (169), age fifty-five years; duration of disease unknown. He was picked up on the street in a confused condition. He had absurd hypochondriacal ideas; "his insides were falling out," etc. He was disoriented and his memory was very poor. The knee-jerks were exaggerated, and the pupils sluggish. There were marked tremors, unsteadiness, and Romberg's sign. His speech was not seriously affected. Depression continued, and he insisted that he could not breathe or urinate, and that parts of him were dead. He died after four months in the hospital. The spinal fluid showed positive lymphocytosis and a positive Wassermann-Noguchi reaction.

E. W. (170), age forty-four years; duration unknown. He had had syphilis. The psychosis was a simple deterioration with marked confusion and disorientation but no delusions. He was silly, his memory was poor, and there were occasional attacks of excitement. The knee-jerks were diminished, and the pupils slightly slow. The speech showed extreme ataxia. Romberg's sign was present, and tremors were prominent. There were frequent convulsions. Death occurred after two months in the hospital.

E. R. (230), age forty-eight years; duration unknown. When admitted she was much demented, confused, completely disoriented, and her memory was poor. Her mood was one of silly elation. The knee-jerks were exaggerated, the pupillary reaction slow and of narrow range. There was marked distortion of speech and writing. There were coarse tremors and increasing ataxia. The spinal fluid showed marked pleocytosis. Death occurred after six months in the hospital.

M. M. (235), age forty-two years; duration uncertain. She had been blind from optic atrophy for two years before admission and had experienced occasional visual hallucinations, but definite mental symptoms seem to have begun only a few weeks before commitment, when she began to show a change of disposition and became forgetful. Syphilis probably occurred twenty years previously. Her husband was a paretic. On admission she was excited, resisting, and obscene. She seemed clear and gave correct answers in the orientation test but her memory showed marked discrepancies in time relations. The knee-jerks were absent, the pupils rigid, her speech drawling and distorted, no tremors, and only slight ataxia. The spinal fluid showed extreme lymphocytosis. She quieted down, and during the next two years showed a gradual deterioration without any particular mental trend. Ataxia increased steadily; she had occasional apoplectic attacks and convulsions. Death occurred after two years in the hospital.

G. F. (236), age sixty years; duration before admission, one month. He became gradually childish, forgetful, and irritable. When admitted he was disoriented, and his memory was poor. The knee-jerks were absent, and the pupils unequal and slow; his speech was slurring and distorted. There were marked tremors and Romberg's sign. He deteriorated rapidly and died after four months in the hospital.

M. D. (242), age thirty-seven years; duration eight months. The onset seemed to date from an injury to the head. There was a probable history of syphilis. He was depressed, confused, and had attempted suicide. His memory was poor, and he was much demented. The knee-jerks were exaggerated; and the pupils unequal and Argyll-Robertson sign present; his speech was ataxic. There were tremors and occasional convulsions. He died after four months in the hospital. The total duration of the disease was one year.

F. B. (299) (figure 1), age fifty-eight years; duration before admission, one month. There was a probable history of syphilis. He was depressed and whining, and had typical paretic ideas, such as that his bowels never moved, and that he had an incurable disease. He would point to a leg or an arm and say, "See, it's all dead, all gone,—there's no hope." His orientation and memory showed marked defects. The knee-jerks were slightly exaggerated, the pupils slow, his speech was distorted, and tremors were excessive. The spinal fluid showed pronounced lymphocytosis and a positive Wassermann-Noguchi reaction. Death occurred after eleven months in the hospital.

Naturally the first question that arises is, Are these not cases of cerebral syphilis in the narrower sense? In reply to this we can but review briefly the differential points. They all showed, in their clinical course, a diffuse, progressive deterioration in all the mental fields, and not the *demence lacunaire* so often observed in syphilitic brain disease. There were no cranial symptoms or other focal manifestations. These points are admittedly not convincing, for it is possible for an old syphilitic endarteritic-meningitic condition to

simulate paresis so closely as to be indistinguishable by its clinical features from the latter disease. It is upon the post-mortem findings that the diagnosis must depend. Anatomically the brains of our cases showed the usual pial thickening, more marked over the frontal convexity. In two it was also prominent over the gyri recti, cerebellum, and cisterna, but it did not assume the degree of meningitis usually seen in syphilis of the base. In one case (235) there was an old hemorrhagic membrane beneath the dura; in another (131) there was a marked preponderance of the paretic process in the right hemisphere, but these conditions were not represented clinically and the findings were otherwise typical. In the microscopic examination the meningeal process in all cases was diffuse, being more marked in the frontal region. The vessel infiltration was also diffuse and found at all depths of the cortex and in the marrow. Plasma cells were, in each case, numerous and usually outnumbered the lymphocytes. Red cells were always present. The nerve cell alterations and changes in the neuroglia do not present differential characteristics and will not be enumerated. With regard to the vessels, a number of our cases, as in all general paralysis material, showed endarteritic changes. In six there was definite thickening of the vessel walls of the type described as syphilitic. In no instance did the intimal proliferation embarrass the lumen to any extent. In the remaining cases the vessels appeared normal. It seems hardly possible that the presence of the spirochætæ could be traced to the luetic endarteritis, since they were not found associated, at least with those vessels which are visible with the Levaditi stain. In none of the cases were there softenings, and no gummata were found either in gross or microscopically.

The spirochætæ were found in all layers of the cortex with the exception of the outer, or neuroglia layer. One was located at the border of this layer, but not within it. A few were found subcortically. Careful search of the pia failed to reveal any of the organisms. In all instances they seemed to have wandered into the nerve tissue. They were not found in the vessel sheaths and seldom in close proximity to the larger vessels. There seems to be no ratio between the number of spirochætæ and the severity of the

paretic process, although the case in which they were most numerous showed excessive paretic changes.

Whether or not, by improving the technique, *Treponema pallidum* can be demonstrated in a much higher percentage of paretic cases will be determined by further investigations.

EXPLANATION OF PLATE 50.

FIG. 1. *Treponema pallidum* in the cortical layer of the right frontal area of the brain (case 299). Stained, with slight modification, by Levaditi's method. × 1,100.

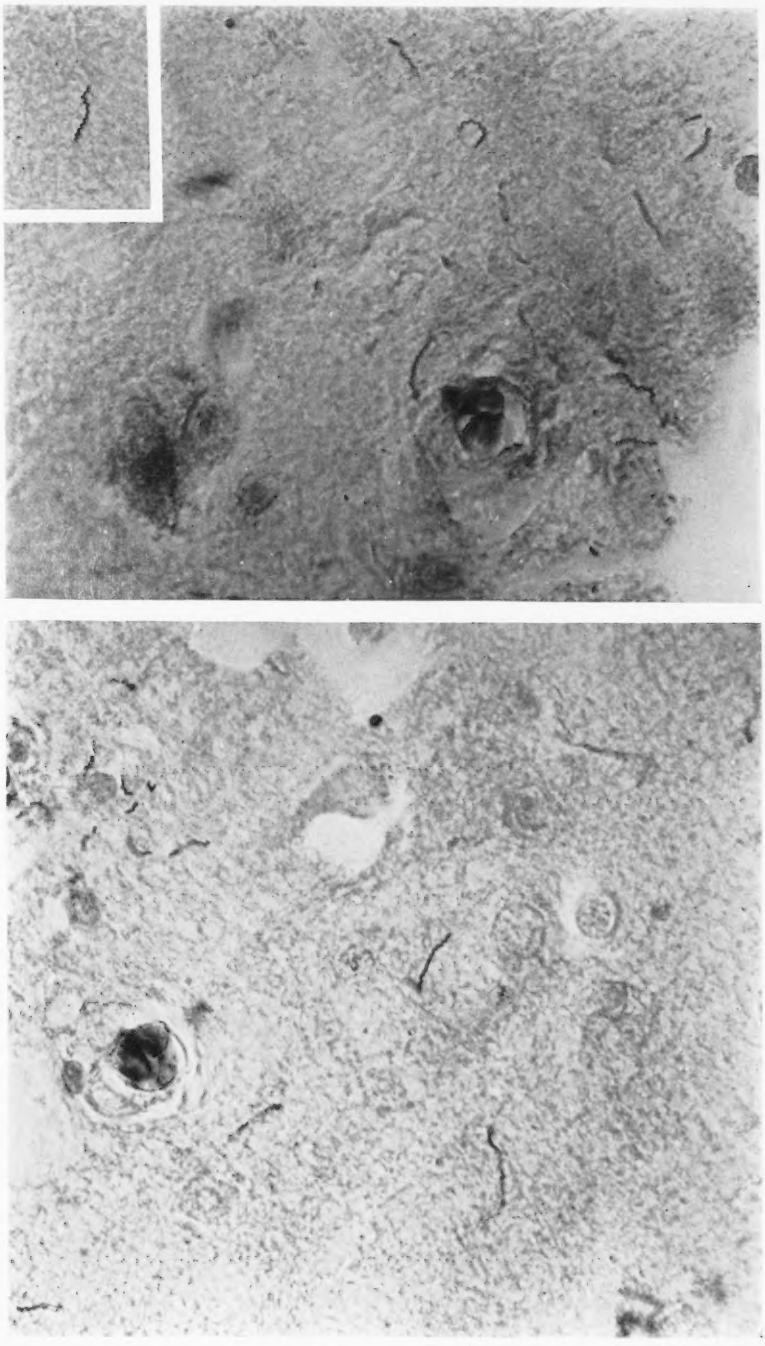

FIG. 1.

(Noguchi and Moore: *Treponema pallidum* in the Brain of General Paralysis.)